

INDIAN COUNCIL OF MEDICAL RESEARCH
TECHNICAL REPORT OF THE
SCIENTIFIC ADVISORY BOARD FOR THE
YEAR ~~xxxx~~ 1953

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TECHNICAL REPORT
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FOR THE YEAR
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I. COMPOSITION OF THE SCIENTIFIC ADVISORY BOARD.

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Dr. D. Soman, Assistant Director, Haffkine Institute, Parel, Bombay.
Dr. R. Veerarahavan, Director, Pasteur Institute of Southern India, Coonoor.
Dr. K. V. Venkatraman, Serologist and Chemical Examiner to the Government of India, School of Tropical Medicine, Calcutta.
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Dr. Nils P. V. Lundgren, Professor of Industrial Hygiene, All-India Institute of Hygiene & Public Health, Calcutta.
Shri N. S. Mankiker, Chief Adviser on Factories, Government of India, Ministry of Labour, New Delhi.
Dr. K. K. Mathur, Medical Officer-in-Charge, Industrial Health Organization, 7/198, Swaroop Nagar, Kanpur.
Lieut.-Colonel M. B. Menon, Assistant Director of Medical Services, Ordnance Factories, Calcutta.
Dr. S. Roy, Chief Sanitary Officer, Mines Board of Health, Asansol.
The Professor of Sanitary Engineering, All-India Institute of Hygiene & Public Health, Calcutta.
Dr. M. N. Rao, Assistant Professor of Physiological and Industrial Hygiene, All-India Institute of Hygiene & Public Health, Calcutta. (Secretary).

XI. Pharmacology.

Bt.-Colonel R. N. Chopra, Director, Drug Research Laboratory,
Jammu, Tawi. (*Chairman*).

Dr. M. D. Chakravarty, Director, Central Drugs Laboratory, Calcutta.

Dr. B. B. Dikshit, Surgeon-General with the Government of Bombay,
Bombay.

Dr. B. N. Ghosh, Professor of Pharmacology, R. G. Kar Medical College,
Calcutta.

Dr. G. K. Karandikar, Professor of Pharmacology, Medical College, Baroda.

Dr. B. Mukerji, Director, Central Drug Research Institute, Lucknow.

Dr. B. B. Yodh, Professor of Medicine, Grant Medical College, 18, Darab-
shah Road, Bombay.

Dr. G. Werner, Professor of Pharmacology, School of Tropical Medicine,
Calcutta.

Shri P. M. Nabar, Drugs Controller (India), Directorate-General of Health
Services, New Delhi. (*Secretary*).



III. TECHNICAL REPORT OF THE RESEARCHES CARRIED OUT DURING THE YEAR 1953.

The researches carried out during the year under report were recommended by the Scientific Advisory Board at its meetings held in Jaipur on the 3rd and 4th December, 1952 and were approved by the Governing Body of the Indian Council of Medical Research at its meeting held in New Delhi on the 28th March, 1953. *The views expressed by the individual workers are not necessarily the views of the Council.*

CHOLERA

1. Inquiry on the immunological studies of *Vibrio-cholerae* under the Director, Central Research Institute, Kasauli.

The purpose of this inquiry is to study questions relating to the immunizing efficiency of cholera vaccines with particular reference to the establishment of international reference preparations for the assay of the potency of cholera vaccine.

At the request of the Biological Standardization Committee of the W.H.O., tests were carried out on the comparative protective value of different samples of cholera vaccines of unknown origin received from the State Serum Institute, Copenhagen. The methods used for the evaluation of the potency of these vaccines were :—

1. Active immunization
 2. Passive immunization
- } of mice followed by test infection with a virulent strain of *V. cholerae*.
3. Assessment of vibriocidal titre after immunization with two doses of vaccine.
 4. Serological examination of vaccines using mono-specific Ogawa, Inaba and cholera rough 'O' sera.

The results of tests brought out the following interesting facts (*see* Tables I to IV) :—

Two vaccines (Nos. 144 and 145) out of the eight tested can be weeded out as unsatisfactory by serological examination alone, provided cholera rough 'O' serum is employed for the purpose. Vaccine No. 144 has been prepared from a strain which is in the smooth-rough phase and vaccine No. 145 has been prepared from a strain which is almost entirely rough. These vaccines evoke poor vibriocidal response in guinea-pigs and afford poor protection to mice as evidenced by active and passive immunity tests. The other six vaccines, Nos. 141, 142, 143, 147, 73849 and 73850, appear to have been prepared from smooth strains of *V. cholerae*; they pass the serological tests, evoke good vibriocidal response and afford considerable protection to mice. In our opinion, these vaccines are satisfactory products for practical purposes. It is interesting to note that vaccines which evoke poor vibriocidal response, Nos. 144, 145, are also poor in affording (a) passive, and (b) active immunity to mice and further that such vaccines can be rejected as unsatisfactory by serological tests involving the use of cholera rough 'O' serum.

TABLE I.

Active immunity tests of cholera vaccines.

Experimental animals, Swiss mice, 17 g. to 22 g. in weight.

Challenge dose at two different test levels, 10^{-3} and 10^{-4} given intraperitoneally 10 days after second immunizing dose.

Period of observation, 72 hours.

Immunizing dose, two doses of vaccine at weekly intervals subcutaneously.

Total volume of challenge dose equal to 0.5 c.c. in 5 per cent mucinized suspension.

Ogawa vaccines.

Vaccine number	Challenge strain	Challenge dose	TOTAL OF TWO DOSES OF VACCINE INJECTED (c.c.)						Controls un-vaccinated	Viable count
			0.16	0.04	0.01	0.0025	0.000625	0.00015625		
142	Ogawa strain 425/52	⁻³ 10	3/6	3/6	4/6	1/6	2/6	2/6	⁻² 10 0/6	15,480,000
		⁻⁴ 10	3/4	5/5	6/6	6/6	6/6	4/6	⁻³ 10 0/6	1,548,000
		⁻³ 10	2/6	2/6	1/6	0/6	0/6	0/6	⁻⁴ 10 0/6	154,800
⁻⁴ 10		5/6	4/6	3/6	2/5	0/6	1/6	⁻⁵ 10 4/6	15,480	
147		⁻³ 10	6/6	6/6	5/6	5/6	2/6	2/6	⁻⁶ 10 4/6	1,548
		⁻⁴ 10	6/6	6/6	6/6	5/6	4/5	4/6	⁻⁶ 10 6/6	
		⁻³ 10	5/6	6/6	6/6	5/6	3/6	2/6	...	
73849		⁻⁴ 10	6/6	6/6	6/6	5/6	5/6	5/6		

Inaba vaccines.

141	V. cholera sub-type Inaba 375/52	⁻³ 10	5/6	4/6	2/5	2/4	2/4	2/6	⁻² 10	0/6	18,700,000
		⁻⁴ 10	6/6	5/6	6/6	5/6	6/6	4/6	⁻³ 10	0/6	1,870,000
142		⁻³ 10	6/6	5/6	6/6	3/6	5/6	0/6	⁻⁴ 10	2/6	187,000
		⁻⁴ 10	5/6	6/6	6/6	5/6	5/6	4/6	⁻⁵ 10	6/5	18,700
145		⁻³ 10	0/5	1/6	0/6	0/6	0/6	0/6	⁻⁶ 10	6/6	1,870
		⁻⁴ 10	2/6	2/6	2/6	2/6	0/5	0/6			
73850		⁻³ 10	2/6	2/4	2/6	1/6	0/6	0/6			
		⁻⁴ 10	5/6	6/6	5/6	4/6	6/6	4/6			

Numerator/denominator = Survivals/total.

From the above findings it is obvious that the serological examination of cholera vaccines yields valuable information regarding the quality and suitability of the products for immunization purposes. The more elaborate active and passive immunity tests do not furnish any additional information which is not obtained from the serological examination alone. We do not wish to decry the use of active or passive immunity tests in assays of relative potencies of cholera vaccines or in the selection of strains for use as seed for vaccine manufacture, but, in our opinion, for routine assays of cholera-vaccine brews, prepared from approved smooth strains, the simple serological tests are adequate. We make this statement keeping in view the practical difficulties that many manufacturing concerns and laboratories will experience, if we insist on certain minimum requirements for anti-cholera vaccine in terms of active and passive immunity units. The use of animal tests, as a routine, would be advisable if by doing so one could obtain extra information of value which could not be got by serological tests, but frankly we are not convinced that it is so. The results of the planned collaborative assays of the vaccines sent by the Department of Biological Standardization, Copenhagen, have convinced us of the soundness of the practice which we follow at this Institute, viz. the use of serological tests only for 'passing' of finished brews of anti-cholera vaccines and the use of both serological and animal tests for selection of cholera strains to be used as seed for mass manufacture of vaccine.

Our minimum requirements at this Institute for (a) passing the vaccines as satisfactory for prophylactic purposes, and (b) for selection of a strain of *V. cholerae* to be used as seed for vaccine manufacture are as follows:—

(a) 1. The vaccine must be prepared from smooth strains. It must contain both Inaba and Ogawa sub-types in equal proportion.

2. The vaccine must agglutinate with Inaba and Ogawa 'O' type specific sera.

3. The vaccine must not agglutinate with cholera rough 'O' serum.

4. In case of vaccines prepared from agar, the vibrio content must not be less than 8,000 million vibrios per c.c.

(b) A smooth strain of *V. cholerae* (G and V 'O' group I) isolated during the course of an epidemic must be used as the seed. It must agglutinate to full titre with the corresponding type-specific 'O' serum. The vaccine prepared from the strain must evoke a satisfactory vibriocidal response in guinea-pigs and afford protection to mice against intraperitoneal infection with a heterologous strain of *V. cholerae*. The results of active (and/or passive) immunity tests obtained must compare favourably with those of the control vaccine maintained at the Institute as reference preparation for the purpose.

Our findings and comments have been communicated to the Expert Committee on Biological Standardization of W.H.O. and will form the basis of discussion at their next meeting of the difficult problems involved in the assay and control of cholera vaccines.

II. MONO-SPECIFIC OGAWA AND INABA AGGLUTINABLE SERA.

Type-specific cholera sera prepared at the Central Research Institute, Kasauli, some four years ago and stored at the State Serum Institute, Copenhagen, for distribution as international reference preparations were tested

TABLE II.

Passive immunity tests of cholera vaccines.

Experimental animals, Swiss mice, 17 g. to 22 g. in weight.

Immunizing dose given intraperitoneally 4 hours before challenge in a volume of 0.2 c.c.

Challenge dose given intraperitoneally in a volume of 0.5 c.c. in 5 per cent mucinized suspension.

Period of observation, 72 hours.

Ogawa vaccines.

Vaccine number	Challenge strain	Challenge dose	DOSE OF IMMUNE SERUM INJECTED (c.c.)						Controls un-vaccinated	Viable Count
			0.05	0.025	0.0125	0.00625	0.003125	0.0015625		
143	<i>V. cholerae</i> sub-type Ogawa strain 425/52	⁻³ 10	6/6	6/6	5/6	6/6	5/6	4/6	⁻² 10 0/6	10,750,000
								⁻³ 10 1/6	10,75,000	
144		⁻³ 10	1/6	1/6	1/6	1/6	2/6	1/6	⁻⁴ 10 3/6	107,500
									⁻⁵ 10 6/6	10,750
147		⁻³ 10	6/6	6/6	6/6	5/6	4/6	3/6	⁻⁶ 10 6/6	1,075
73849		⁻³ 10	5/6	6/6	6/6	6/6	6/6	6/6		

Inaba vaccines

141	<i>V. cholerae</i> sub-type Inaba strain 375/52	⁻³ 10	4/6	6/6	6/6	6/6	4/6	5/6	⁻² 10 0/6	16,800,000
									⁻³ 10 1/6	1,680,000
142		⁻³ 10	5/6	6/6	6/6	5/6	6/6	5/6	⁻⁴ 10 5/6	168,000
									⁻⁵ 10 6/6	16,800
145		⁻³ 10	4/6	3/6	1/6	1/6	1/6	1/6	⁻⁶ 10 6/6	1,680
73850		⁻³ 10	6/6	5/6	6/6	6/6	5/6	4/6		

Numerator/denominator = Survivals/total.

for type specificities and keeping qualities. No difference in titre between the samples or between the original tests carried out four years ago and the renewed tests were noticed. The sera keep up their agglutinating capacities satisfactorily under cold storage during long periods as well as during postal transmission. These sera have now been accepted as suitable international reference preparations.

III. ENZYMES OF *V. CHOLERÆ*.

In our report for 1952 we mentioned that an enzyme capable of dissolving collagen was present in culture filtrates of cholero-genic and non-cholero-genic vibrios. Further studies of this enzyme show that it does not act on native collagen and that it is of the nature of a trypsin. *Vibrio cholerae* filtrates containing this enzyme have been found capable of dissolving muscle tissue and proteins of dried vibrio cells.

The presence of elastinase in vibrio-culture filtrates has been confirmed and further work has shown that it is capable of acting both on the native and purified elastin at an optimum pH of 8. The action of this enzyme is inhibited by animal and human sera and by dialysates of ground pancreatic tissue.

The mucinolytic, tryptic and elastinolytic properties of vibrio filtrates as well as their lipase, amylase and invertase activities have been found to be due to different enzymic entities. These properties are not peculiar to *V. cholerae* alone but are exhibited by non-cholero-genic vibrios.

To assess the possible rôle of these enzymes in cholera pathogenesis, the following experiments were carried out: Membrane sacs were made using living pieces of guinea-pig ileum and the effects of vibrio filtrates on permeability of these membranes investigated. It was found that there was a notable increase in permeability when vibrio filtrates were allowed to act on the membranes. Some thermolabile constituent or constituents of the filtrates abolish peristaltic activity. The factors causing the increased intestinal permeability produce their effect independent of the muscular or nervous mechanisms concerned with peristalsis since membranes which have lost their peristaltic capacity are also equally affected by the filtrates. This activity is demonstrable when the filtrate is allowed to act on the intestine from the lumen to the peritoneum or from the peritoneal surface to the lumen. The activity is inhibited by normal serum which contains an elastinase inhibitor as well as a trypsin inhibitor. The ultimate result of the prolonged action of the filtrate—18 hours—on the intestinal wall is disintegration of the tissues.

Histamine 1-500 in Ringer-Locke solution does not produce increase permeability or peristalsis changes when tried under identical conditions.

Filtrates of both *V. cholerae* and NAG vibrios have been observed to give identical results in permeability experiments.

Membranes consisting of the walls of blood vessels also show, like intestinal membranes, increased permeability when acted on by *V. cholerae* filtrates. The effect is observed both when the action takes place on the blood-vessel wall from the periphery to the lumen or from the lumen to the periphery.

TABLE III.

Vibriocidal response to cholera vaccines.

Experimental animals — Guinea-pigs 300 g. to 400 g. in weight.

Immunizing dose—Two doses of vaccine (0.5 c.c. and 1 c.c.) at weekly intervals subcutaneously.

Vibriocidal titre tested 10 days after second immunizing dose.

Ogawa vaccines			Inaba vaccines		
Vaccine number	Vibriocidal titre		Vaccine number	Vibriocidal titre	
	Inaba	Ogawa		Inaba	Ogawa
143	1/30	1/10,935	141	1/10,935	1/10,935
144	1/405	1/135	142	1/10,935	1/10,935
147	1/3645	1/32,805	145	1/15	1/45
73,849	1/3645	1/32,805	73,850	1/32,805	1/32,805

*Method of test—As published in *Indian Journal of Medical Research*, 1948, **36**, p. 3.

TABLE IV.

Agglutination results of cholera vaccine suspensions when tested against mono-specific cholera 'O' sera and cholera rough 'O' sera.

Ogawa vaccines				Inaba vaccines			
Vaccine number	Inaba type 1000	Ogawa type 1000	Cholera rough 1000	Vaccine number	Inaba type 1000	Ogawa type 1000	Cholera rough 1000
143	—	1000	—	141	500	—	—
144	—	500	500	142	500	—	—
147	—	500	—	145	50	50	500
73,849	—	250	—	73,850	500	—	—

2. Investigation on cholera endemicity with special reference to Hilsa and other estuarine fishes under Dr. M. N. Lahiri, at the All-India Institute of Hygiene & Public Health, Calcutta.

Collection of water samples was continued from the surface and bottom of a selected stretch of the river Hooghly, both from the shore and the mid-stream, at three fishing centres, viz. Nawabgunge, Baghbazar and Falta or Diamond Harbour. The last station which is an important endemic centre for cholera was selected when fishing was closed at Falta. A total of 722 samples of water were examined, and out of these, three samples were positive for cholera vibrios and 306 samples for NAG vibrios.

*Ahuja, M.L., and Gurkirpal Singh (1948), Observations on cholera vaccines—*Ind. Jour. Med. Res.* **36**, 1, p. 3.

For the corresponding period last year, seven positive isolations of cholera vibrios were made. All these isolations were obtained from March to May, but the period of occurrence this year was found to extend up to July from March.

As during the previous year, all the isolations were made from the upper reaches of the estuary (Nawabgunge) at high tide only. Two isolations were made from the surface and one from the bottom. Out of the three strains isolated, two were of the Ogawa type and one of the Inaba type. NAG vibrios isolated from water samples were grouped according to Heiberg's classification. Groups I, II and V formed the predominant constituents of the vibrio flora of water. The seasonal fluctuations of groups I and II show a definite correlation with the period of cholera epidemics in Calcutta.

The pH and salinity of the water samples collected from the three fishing centres were determined with a view to elucidate any probable correlation between these values and the occurrence of vibrios. While the fluctuations in pH are not very marked, the salinity varies to an appreciable extent. Besides the diurnal variations due to tidal influence, seasonal variations are caused by the large quantities of flood water brought down during the S.-W. monsoons and the excessive evaporation during the summer months. As is to be expected, the average salinity is higher at the lower reaches of the estuary at Falta and Diamond Harbour than in the upper reaches at Nawabgunge and Baghbazar. It is interesting to note that isolations of cholera vibrios have been made only from the upper reaches of the estuary. No definite correlation appears to exist between the occurrence of vibrios and the salinity fluctuations of the Hooghly water.

The possibility of vibrios being adsorbed by the silt particles in the water and being deposited on the estuarine bottom, where they thrive, was considered to be a fruitful line of investigation. Pending the development of a technique for the concentration of bacterial flora from a large quantity of silt, the top layer of silt from an area of approximately 1 sq. ft. was collected by hand, from the marginal area at low tide, from which about 10 g. were taken at random for bacteriological examination. No *Vibrio cholerae* have so far been isolated, but NAG vibrios have been obtained. Out of a total of 102 samples examined, 44 were positive for vibrios. No distinct relationship between the seasonal fluctuations in the occurrence of the two predominant groups, viz. groups I and II, and the seasons of cholera epidemics could be traced from the available data.

Preliminary experiments were conducted to study the survival of vibrios in silt. Silt collected from the Hooghly river bed was sterilized and crushed into a fine powder. In test-tubes containing 5 g. of this silt, 10 c.c. of water were added and live cultures of AG and NAG vibrios were inoculated into them, keeping suitable controls. Significant differences were not found in the survival of vibrios in the water and the silt, but it is necessary to repeat the experiments on a larger scale to arrive at any definite conclusions.

Different size groups of hilsa ranging from the fry stage to the adult stage were collected during the seasons of their availability in the river Hooghly from the three selected centres every week and their gut-contents and gill-samples bacteriologically examined. During the off-season for Hilsa, other estuarine fishes available were examined. No *Vibrio cholerae* have so far been isolated, but NAG vibrios were obtained. Out of 204

samples of Hilsa, consisting of 648 specimens and 55 samples of other fishes consisting of 268 specimens, the gut-contents of 76 samples of Hilsa and 34 samples of other fishes were positive. The gills of 77 samples of Hilsa and 26 samples of other fishes were also positive for vibrios. NAG vibrios have been found to occur in the fishes, either in the guts or the gills, throughout the year. Groups I, II and V are the three predominant groups of NAG isolated from the alimentary tracts of fishes. The seasonal fluctuations in the occurrence of groups I and II show a definite correlation with the periodicity of cholera in Calcutta. Parallel data for the gill samples also give a picture somewhat similar to the one obtained for the gut-contents. In view of the above relationship it seems that groups I and II vibrios are in some way connected with the maintenance of cholera endemicity.

In the absence of better equipment, the pH of the gut-contents of Hilsa was studied by the spot-test method. The stomach-contents were found to be definitely acidic and the intestinal contents showed a pH ranging between 6 and 7. In view of the possibility that the gut-contents may be undergoing some chemical changes during the period that elapses between the capture of the fish and their dissection in the Laboratory, thus affecting the viability of the vibrios, similar samples of fish were dissected in the field and in the Laboratory. No significant differences were noticed in the pH or the vibrio flora of these two sets of samples.

As a preliminary to artificial infection experiments envisaged in the programme, it was necessary to perfect a method of collection, conditioning and rearing of Hilsa in confined areas. Hilsa is an extremely delicate fish and it was believed that it will die immediately after it strikes the net used for its capture. The settling tanks and filter-beds of the Calcutta Corporation Water Works at Pulta was considered the cleanest and most suitable source from which collections could be attempted. After a good deal of experimentation, it was possible to perfect a method of collecting young Hilsa and rearing them in cement cisterns 10' \times 3' \times 3'. Water from the settling tanks was fed into these cisterns and plankton was provided as fish food.

In view of the possible difficulties in transporting the fish to Calcutta for the artificial infection experiments, it was decided to conduct these in Pulta in cement cisterns at the premises of the Central Inland Fisheries Research Station. Before these experiments are undertaken, it was, however, thought advisable to try some small-scale experiments with hardier fishes in the Laboratory in the form of pilot experiments. Kôï (*Anabas testudineus*) and Lata (*Ophicephalus striatus*) were employed for these studies.

Specimens of these two species were reared separately in vibrio-free water in glass aquaria and fed on vibrio-free food. The water was renewed daily and the old water examined bacteriologically for the presence of vibrios. When the water proved negative for three consecutive days, they were fed on infected food. Pupæ and larvæ of house-flies bred in the Laboratory and fed on vibrio-free milk were injected with live cultures of cholera vibrios and the fish were fed on these. Every day, the fish were removed to fresh sterilized aquaria, after washing them several times in vibrio-free water. The water from the aquaria was examined daily, but no vibrios were recovered for over 30 days. The fish were then dissected and their intestinal contents bacteriologically examined. Hæmolytic non-agglutinable vibrios were recovered.

This result led to an examination of the possibility of the vibrios remaining in the guts without being excreted, as also of the artificially infected specimens having had vibrios in their alimentary tracts before they were infected. After a consideration of possible means of checking up the vibrio-free nature of the gut of a fish before it is artificially infected, it was decided to conduct an experiment to determine this.

A sample of thirty Kôï (*Anabas testudineus*) was reared in a large tub in vibrio-free water which was changed every day. The fish were fed on boiled yolk of eggs regularly. Samples of 1,000 c.c. of water were examined from the tub daily for the presence of vibrios and after they were negative consecutively for over a week, ten specimens were taken at random and dissected to find out if vibrios were present in their guts. One fish showed the presence of inagglutinable vibrios. The experiment is now being repeated with smaller number of fish and lesser quantity of water, so that the whole water in the aquaria can be examined bacteriologically and the possibility of vibrios having remained undetected can be ascertained.

Particular attention was directed to the study of the cholera enzyme, mucinase present in the filtrates of *V. cholerae* which has been reported to cause sloughing of the mucosa from strips of guinea-pigs ileum (Burnet & Stone, 1947). After a good deal of experimentation a method for the quantitative titration of the enzyme has been standardized. It has been observed that mucinase elaboration and the maintenance of quantitative activity are influenced by several factors, i.e. media, pH, moisture, etc.

Twenty-seven strains of *V. cholerae* (21 Ogawa sub-type and 6 Inaba sub-type) isolated from cholera patients and from Hooghly river water during the past two years were tested. With each strain the mucinolytic activity was evident at a titre of 1 in 1,600 or above. One strain, however, showed a very high titre of 1 in 25,600, and a second strain tested on four occasions during a period of six months gave a constant titre of 1 in 6,400. The presence of this enzyme has also been demonstrated in certain intestinal organisms, i.e. *B. coli* type I, *Salm. enteritidis*, and *Sh. flexneri*, but the titre was of a very low order and never exceeded 1 in 10. In the course of this work a smooth strain of *V. cholerae* became rough, and when tested later its mucinase-forming property was found to be very considerably lowered. It is presumed that this is true for all strains of *V. cholerae*, if or when they become rough. Subsequently, investigations were carried out with 18 strains of NAG vibrios. These were isolated from Hooghly water, the gut and gall-bladder of different varieties of fish and also the stools of cholera patients. With the exception of one which gave as a high titre as that of the cholera vibrios, i.e. 1 in 6,400, all showed considerable variation in the elaboration of the enzyme but the titre was low and ranged from 1 in 100 to 1 in 400. Thus, it appears that the intensity of mucinase activity is of greater importance rather than its mere presence.

Since high titres have so far been obtained with true cholera vibrios, it appears that it may be possible to utilize the test as an adjunct to other known tests which characterize such vibrios.

MALARIA.

Insecticide and mosquito repellent inquiry under the Director, Malaria Institute of India, Delhi.

The following investigations were carried out in the Laboratory and field :—

A. LABORATORY INVESTIGATIONS :

1. Solubility of DDT crystals in cuticular wax of house-flies and mosquitoes.
2. Resistant strain of *Culex fatigans*.
3. Susceptibility of DDT-resistant strain of *Culex fatigans* to other chlorinated hydrocarbon insecticides.
4. Effect of feeding normal and resistant strains of *Culex fatigans* on DDT-fed fowls.
5. Rapid method of screening DDT water-dispersible powder samples.

B. FIELD INVESTIGATIONS :

1. Studies on the behaviour of mosquitoes in relation to insecticidal application.
2. Evaluation of DDT, BHC, DDT and BHC combined and dieldrin residual sprays against mosquitoes in different parts of India.

C. CONFIRMATION OF FINDINGS OF THE INSECTICIDE AND MOSQUITO REPELLENT INQUIRY.

D. PUBLICATIONS.

A. LABORATORY INVESTIGATIONS.

1. *Solubility of DDT crystals in cuticular wax of house-flies and mosquitoes*—Pal, (*Bull. Ent. Res.*, **41**, 1, pp. 121-139, 1950) studied the wetting of insect cuticle by insecticidal liquids ; as the effectiveness of almost all contact insecticides depends upon it. He found that apart from the chemical and physical nature of the cuticular lipoids, irregularities on the body surface were important. With the application of residual deposits of insecticides, however, the position is completely different. The insecticide is not available in the form of liquid but as crystals. Under such circumstances the rate and degree of dissolution of crystals in the cuticular wax is an important factor affecting the toxicity of contact insecticides. The object of the present investigation was to study the solubility of DDT in the cuticular wax of insects particularly those of public health importance.

Cuticular wax of *Musca nebulo*, *Anopheles stephensi* and *Culex fatigans* were extracted in chloroform by soaking a large number of insects in sufficient quantity of chloroform for 24 hours. The lipid-chloroform solution was filtered through a cintered glass-funnel and the chloroform was allowed to evaporate at room temperature. One c.c. of 1 per cent solution of cuticular wax in chloroform was uniformly applied on the excavated portion of a cavity glass-slide (area about 2.27 sq. cm.). Technical DDT specially pulverized was dusted on the wax-treated area of the slide after ensuring complete evaporation of chloroform.

Micro-photographs of the crystals on wax were taken without disturbing the slide, after 5 and 30 minutes, 1, 3, 6 and 24 hours.

It was observed that the dissolution of crystals in the cuticular wax of *Musca nebulo* and *Anopheles stephensi* started almost immediately and they almost completely disappeared in 3 and 6 hours, respectively. In the case of *Culex fatigans* crystals were observed intact even after 24 hours. This is of considerable interest because these observations are in conformity with the natural biological resistance of *Culex fatigans* to DDT residual deposits.

2. *Resistant strain of Culex fatigans*.—Reports of culicine mosquitoes having developed resistance to DDT have been received from several parts of the world. In those states of India where DDT residual spraying has been in progress for the last six years or so, there is a possibility of certain culicine species having developed resistance to DDT. Investigations were undertaken in Delhi State where DDT has been in use since 1946. *Culex fatigans* were captured from a sprayed village Khuraiji Khas which had been regularly sprayed with DDT during the past six years, and from an unsprayed village Ram Garhi (U.P.) 10 miles apart. These two strains were reared for one generation simultaneously under identical conditions. The progeny of the strains was tested for resistance to DDT. The mosquitoes were exposed in glass-bottles ($2\frac{3}{4}$ " diameter and 7" high) coated with triple crystallized DDT in acetone at the rate of 300 mg. per sq. ft. The results indicated increased resistance of mosquitoes to DDT. Cent per cent knock-down of mosquitoes collected from unsprayed village was obtained within five hours, whereas only 66 per cent (females) and 79 per cent (males) mosquitoes from sprayed village were knocked-down during that period. Even after 24 hours contact, only 78 per cent (females) and 24 per cent (males) mosquitoes were knocked-down. In subsequent tests the mosquitoes were exposed for half-an-hour to glass-panels sprayed with DDT at the rate of 50 mg./sq. ft. The exposed insects were kept under observation for 48 hours. Twenty experiments were carried out, mortality amongst the mosquitoes from sprayed village was nine per cent (females) and 31 per cent (males) after 48 hours, and the corresponding figures for the mosquitoes from unsprayed village were 90 and 93 per cent, respectively.

3. *Susceptibility of DDT-resistant strain of Culex fatigans to other chlorinated hydrocarbon insecticides*.—One object of this investigation was to determine whether or not DDT-resistant strain of *Culex fatigans* was also resistant to other chlorinated hydrocarbon insecticides. Both normal and resistant strains were exposed to glass-panels treated with DDT, chlordane, dieldrin applied at the rate of 50 mg./sq. ft. and BHC gamma isomer at the rate of 10 mg./sq. ft. for 5 and 15 minutes, and were then kept under observation in clean cages for 24 hours after which the mortality was recorded. It was found that the mortality rate was comparatively low when the resistant strain was exposed to DDT, whereas in the case of other insecticides the mortality rates were high and almost identical to those of the normal strain. It seems that the DDT-resistant strain of *Culex fatigans* is initially not cross resistant to other insecticides tested.

4. *Effect of feeding normal and resistant strains of Culex fatigans on DDT-fed fowls*.—DDT was fed to fowls at the rate of 100 mg. per kg. of the body-weight for three days in succession. On the fourth day they were exposed to the normal and resistant strains of *Culex fatigans* and other species to provide

blood feeds simultaneously. Broadly speaking, the reactions of the mosquitoes were similar in pattern after imbibing blood meal from a DDT-fed fowl as after exposure to a dry film of DDT. Thus, while no death occurred in the resistant strain of *Culex fatigans*, there was significant mortality in the normal strain in which mosquitoes died only during the first two days and not thereafter when blood meal had been digested and ovaries had matured.

Susceptible species, like *Aedes aegypti*, were very adversely affected. The engorged females developed severe symptoms of DDT poisoning which resulted in mortality up to 100 per cent within 24 hours.

The method of feeding mosquitoes on DDT-fed fowls as outlined seem to provide a reliable means of assessing the widely varying susceptibility of different species of mosquitoes and of evaluating the degree and level of resistance of different strains of the same species.

5. *Rapid method of screening DDT water-dispersible powder samples.*—The planning body of the National Malaria Control Programme (N.M.C.P.) after careful scrutiny decided to recommend the use of 75 per cent DDT water-dispersible powder and 4,000 tons of this formulation was imported in different consignments at the ports of Bombay, Madras and Calcutta during the current year. On receipt of 1952 supplies, Mysore State reported that the water-dispersible powder resulted in a very poor suspension when mixed with water. It was, therefore, decided that before any fresh receipts were despatched to the States it was necessary to test them, especially with a view to assess its compliance with the specifications laid down by the WHO (WHO Technical Report series, No. 54, p. 16). These specifications pertain to the chemical and physical requirements, particle size, agglomeration, wettability, suspensibility, acidity, tropical storage, etc. Tests on the most important of the physical properties, viz. suspensibility, only were carried out as preliminary tests on wettability were satisfactory. Qualitative tests described below were substituted for quantitative tests indicated by the WHO :—

Each shipment consisted of supplies from different manufacturers. Drums were selected at random from supplies made by each manufacturer. 3.3 gms. of sample were accurately weighed and mixed with hard water to make up to 100 c.c. in a 100 c.c. graduated cylinder. For proper mixing, the cylinder was inverted 30 times, after which it was allowed to stand for 15 to 30 minutes. If more than 10 c.c. by volume of this powder settled down within 15 minutes it was considered as unsatisfactory. If, however, the settling of the powder was less than 10 c.c. in 15 minutes it was considered satisfactory for all practical purposes.

With this rapid procedure, it was possible to test samples from as many as 53,342 drums within a short time before the supplies were despatched for use in different States.

B. FIELD INVESTIGATIONS.

1. *Studies on the behaviour of mosquitoes in relation to insecticidal applications.*—Field studies on the behaviour of mosquitoes in relation to insecticidal application have been in progress since 1950. These were concluded during the period under report and data analysed. The main conclusions arrived at are as follows :—

(a) Study of the entry and feeding activity of mosquitoes and their movements in comparison and insecticides-treated huts have shown that

insecticidal application does not alter the normal behaviour of the species studied.

(b) Excito-repellent effect was observed in mosquitoes after contact with surfaces treated with DDT suspension and emulsion at the rate of 50 mg. DDT/sq. ft.

(c) BHC deposits at the rate of 10 mg. gamma isomer per sq. ft. also exerted repellent effect on mosquitoes.

(d) The repellent or excito-repellent effect of DDT and BHC deposits on mosquitoes is of little significance as regards their control is concerned because in both cases the mosquitoes pick up lethal dose and eventually die.

2. *Evaluation of DDT, BHC, DDT and BHC combined and dieldrin in different parts of India.*—To evaluate the different insecticides against mosquitoes under different field conditions, studies have been undertaken in the villages of Punjab, Uttar Pradesh and Bombay. Field studies in Punjab and U.P. are being conducted by the staff of the Indian Council of Medical Research and the Malaria Institute of India in co-operation with the State authorities concerned. Both entomological and epidemiological data are being collected which would be analysed when the studies are completed. The following insecticides used in form of suspension are being tested at different dosages:—

1. DDT 50 mg./sq. ft.
2. Dieldrin 6.25 mg./sq. ft.
3. Dieldrin 12.5 mg./sq. ft.
4. Dieldrin 25 mg./sq. ft.
5. BHC 10 mg. gamma isomer/sq. ft. (I.C.I. 'Gammexane' P520, imported).
6. BHC 10 mg. gamma isomer/sq. ft. (I.C.I. 'Gammexane' P520, Indian made).
7. BHC 10 mg. gamma isomer/sq. ft. (Hexidol-Geigy).
8. DDT 25 mg./sq. ft. and BHC 5 mg. gamma isomer/sq. ft.

Gross toxic hazards of dieldrin to spraymen and the inhabitants of treated villages are being studied in Delhi State, where number of villages with a total population of about 10,000 have been sprayed with dieldrin (25 mg. sq. ft.). Spraymen are weighed and physically examined before and after each round of spray. Similarly, groups of about 100 children each from DDT and dieldrin-treated villages are under examination to determine any apparent toxic manifestations.

(C.) CONFIRMATION OF FINDINGS OF THE INSECTICIDE AND MOSQUITO REPELLENT INQUIRY.

(i) Synergistic effect of DDT and BHC combined spray against mosquitoes was reported in 1950. Investigations by other workers (Dicke, R. J. and Paul, J. J., *Jour. Econ. Ento.*, **44**, pp. 896-898, 1951; Van Tiel, N., *Bull. Ents. Res.* 1952, under publication) and Davidson, G. (1953) *Bull. Ent. Res.*, **44**, pp. 231-54 have now confirmed these findings and they have also reported the superiority of DDT and BHC combined spray over either of them applied separately. In Bombay State (Viswanathan, D. K., *Bull. Nat. Soc. Ind. Mal. Mosq. Dis.*, **1**, pp. 57-69 1953), on the other hand, combination of DDT and BHC did not yield any demonstrably better results. These results along with the data have, however, not been published so far.

(ii) Since 1950, in the field experiments with different insecticides against mosquitoes, total mosquito-catch data from sprayed and unsprayed catching stations of the treated villages was found very useful in evaluating residual sprays (Pal, R. *Ind. J. Mal.*, **5**, pp. 195-199, 1951. Entomological observations from comparison village may sometime vary from the experimental villages due to some natural causes and hence are not always helpful in the assessment of results. Other workers (Rao, B. A. 1953, *personal communication*) have also expressed similar opinion and they also consider that entomological data from a comparison village is unnecessary ; though it may provide useful epidemiological data.

(iii) Resistance in terms of knock-down or mortality ; Pal and Sharma (1951, *Proc. IXth Internat. Cong. Entomol.*) and Pal *et al.* (*Ind. Jour. Malariol.*, **6**, pp. 303-316 1952), in their studies on the development of resistance to insecticides in houseflies concluded that resistance of flies, should be specifically mentioned in terms of ' knock-down resistance ' and ' mortality resistance ' because wide variations exist between the two. These findings have been confirmed by Busvine, J. R. (*Nature*, **171**, pp. 118-119, 1953).

D. PUBLICATIONS.

2. Inquiry to study nutritional states and their effects on mammalian malaria under the Director, Malaria Institute of India, Delhi.

The object of the inquiry is to determine the effect of different nutritional states of the host (rats) on the host-parasite relationships of animals with *P. berghei* infections.

It has been stated in the last year's report that multiplication of this parasite in starved host was not of the order as in normally fed hosts. Three possible reasons were considered, namely an increased resistance in the host to invasion of parasites, starvation of parasites themselves and ketosis in the host. The first possibility was ruled out as the parasites multiplied normally in infected hosts which were fed after a certain period of starvation. The second possibility was perhaps the most probable cause of infection not establishing itself during starvation. Infected animals were fed on PABA during starvation and the parasites became patent though their density was low. This seemed to show that PABA is probably one of the essential nutrilites for *P. berghei* and that it is not readily available in sufficient quantity to the parasites during starvation of the host. Similar experiments during the current year showed that methionine is another essential nutrilitite not available during starvation.

Thirdly, a highly ketogenic diet consisting of a high proportion of butter-fat was given to infected animals. The parasitaemia was less than in controls fed on normal diet and showed the probability of ketosis being an additional factor for the restricted parasitic growth in starved hosts.

The age of the animal appears to be important in the consideration of its host-parasite relationship during its starvation. In two experiments it was found that when six-week old infected animals were starved, the parasites were patent in considerably higher numbers and for a longer period than in similarly treated adult animals. The high susceptibility of young animals to infection seems to persist even when they are starved.

Feeding infected and starved hosts on chemically pure nutrilites, either singly or in combination, has led to a better understanding of the

physiological needs of parasites. The scope is wide in respect of vitamins, particularly with the availability of specific vitamin antagonists.

EFFECT OF DIFFERENT QUANTITIES OF THE SAME DIET ON THE COURSE OF INFECTION IN ALBINO RATS

Six replicate experiments were carried out in 108 rats. The experimental animals were given half the quantity of standard diet for control animals.

Under-nourishment, in so far as malaria is concerned, bears a two-fold effect: one on the malaria parasites and the other on the host's defence mechanism. The parasite in the under-nourished host grows and multiplies to a less extent than in a well-nourished host. An interesting feature is that due to impaired capacity of the host to develop immunity even mild infection tends to end fatally.

Under-nourishment seems to greatly affect the capacity of the host to acquire immunity. If an infected under-nourished (or starved) host is fed normally the parasite reaps the benefit more quickly than the development of host-defence mechanism. Under such circumstances, the parasite multiplies rapidly both due to better available nourishment as well as the absence of adequate check by host immunity. More often than not the host dies. Such a sequence of events probably explains the high case mortality during malaria epidemics and in particular the one which occurred during the Bengal famine of 1943. It was noticed that institution of relief measures, at a time when fresh transmission was low or absent, resulted in greater number of deaths due to malaria.

EFFECT OF DIFFERENT QUALITY DIETS ON THE COURSE OF INFECTION IN RATS

One hundred and fifty rats were used for the study which consisted of six experiments, some of which were replicates. The animals in each group, experimental as well as control, were mostly of the same age and sex distribution.

The different diets used in the experiments were of three types: representing vegetarian, lacto-vegetarian and mixed. It was ensured that these diets were not deficient in any of the vitamins or minerals.

Vegetarian diets of a high-carbohydrate content, containing rice or wheat gave, rise to a more severe acute infection than normal balanced diet. Very few of the animals on such a diet died of chronic infection and the degree of acquired immunity was sufficient to nullify a challenge inoculation. Lacto-vegetarian diets consisting of milk and rice or wheat were responsible for a milder acute infection as compared to vegetarian diet, though the course of chronic infection was similar in animals on either of the above diets.

Animals on a diet with a high proportion of meat suffered from a more severe, acute as well as chronic, infection than those on other diets. It seemed logical that it should be so in view of the fact that such a diet probably provided, in abundance, all nutrients required by the parasite, far in excess of its capacity to enhance the acquirement of immunity by the host.

Among the different proteins constituting these diets, milk protein more clearly established a host-parasite relationship in favour of the host.

The course of *P. berghei* infection in rats on an exclusive milk diet was milder than in those fed on balanced diets. This was due to some deficiency in the diets which did not provide *P. berghei* with all its physiological requirements. The diametrically opposite effects of milk diet on *P. gallinaceum* in fowls (*Ramakrishnan, *et al.*, 1953) seem to emphasize the specificity of physiological characteristics of plasmodial species and perhaps of hosts also.

PYRIDOXINE (VITAMIN B₆) DEFICIENCY AND MALARIA

Twelve albino rats were fed on three diets, a balanced diet, one deficient in pyridoxine, and the other fortified with pyridoxine. The animals were pre-conditioned on these diets for a month and then inoculated.

The acute infection in rats fed on pyridoxine-deficient diet was mild as compared to that in the controls. The daily average parasitaemia in the above three diet groups was of the ratio 11 : 1 : 4, respectively. Similarly, peak parasitaemia in them was of the ratio 7 : 1 : 3. No mortality occurred from acute infection in the deficient group, while it was 100 per cent in the two control groups.

It appeared that pyridoxine was also an essential requirement of *P. berghei*. The deficiency did not seem to appreciably affect the immunity mechanism of the host.

3. Inquiry on chemotherapy of malaria at the School of Tropical Medicine, Calcutta.

A number of compounds synthesized had potent action on blood-induced infections of *P. berghei* in mice, but none was superior to the appropriate standard reference compounds. One of the test compounds was also active against *P. gallinaceum* in Terzian's prophylactic mosquito test. As this compound, a complex biguanidine (ref. No. MM5), appeared to be less toxic to mice than proguanil, it was given further trials.

Further tests with sulphadiazine on *P. berghei* infections were made in order to determine its m.e.d. for the purpose of evaluating the activity of some of its derivatives and other sulphonamides. Its m.e.d. is 0.075-0.1 mg./kg., yielding a Q.E. of 600-800. Six derivatives of sulphadiazine were screened : four of these derivatives tested in an arbitrary dose of 800 mg./kg. for comparison with other sulphonamides previously tested were curative. All six were then tested for determination of the m.e.d., commencing with a dose of 1 mg./kg. The compounds were highly active, the best of them, cinnamalydine sulphadiazine, showing activity at 0.1 mg./kg. Acute oral toxicity tests with the latter derivative and sulphadiazine showed that the two bases were not toxic at 2 mg./kg. A soluble salt of the derivative is required for toxicity tests at higher doses for comparison with sodium sulphadiazine. Four thiazoles and one pteridine showed little or no activity in doses ten or more times the m.e.d. of proguanil. MM5 was given further trial and the results were disappointing, the compound showing less activity and more toxic effects than in the first trials last year. The explanation for this variability is not precisely known, but it is thought that old samples of the compound lose potency in some manner. Using a fresh and carefully prepared sample, approximation to the best results was, however, obtained, though the curative effect of

* *Indian Journal of Malariology* (1953), 8, (in press).

40 mg./kg. was not confirmed. Parallel experiments with MM5 were done in mosquitoes infected with *P. gallinaceum* and here too the compound gave variable results which tended to confirm suspicions with regard to its stability. In the final trials with the fresh sample, the excellent results obtained last year were fully reproduced, and it was concluded that the compound merited trial against the human plasmodia in *Anopheles*. Cinnamalydine sulphadiazine proved to be as active as the parent compound in a test on *P. gallinaceum* in mosquitoes, both being active in 0.1 per cent concentration but not in 0.01 per cent.

The inquiry was transferred to Calcutta in May 1953, for the purpose of testing MM5 against *P. vivax* in *Anopheles stephensi*. A laboratory colony of *A. stephensi* derived from a single captured specimen was established. In view of past experience with the compound, a few preliminary tests against *P. gallinaceum* in *Aedes aegypti* were carried out in order to ensure that the sample of MM5 specially prepared for the tests on the human plasmodia was potent. The sample was found to be active. Only one experiment with *P. vivax* could be carried out in the time available after the preliminaries. The result suggested activity in that no gland infection developed in the test group, while 62 per cent of controls showed gland infection. It was, however, noted that the controls also showed abnormal oöcyst development, many of the oöcysts appearing degenerate. The mosquitoes as a whole in this experiment appeared unhealthy and it is probable that this was due to unfavourable climatic conditions prevailing at the time of the experiment which was made under laboratory conditions during the height of the monsoon. MM5 was tested in 0.01 per cent concentration and its toxic effects on the mosquitoes were probably intensified by the unfavourable environment. More marked degeneration of oöcysts with chitination and 'black-spore' formation was observed in an experiment with *P. gallinaceum* made at the same time. Hence, no definite result was achieved as to the activity of the compound against *P. vivax*.

4. Inquiry on the effects of surface and surface treatment on the residual toxicity of various formulations of DDT with special reference to conditions in rural India under Shri J. C. Vedamanikkam at the Central Malaria Laboratory, Madras.

The walls of most of the human dwellings in malarious areas are made up of mud and clay and finished with cowdung or lime or charcoal according to local and community differences. The roofs are usually made up of leaves of palmyra or coco-nut palm or straw or grass whichever is available. Below is given a report on the study carried out on the differences in the chemical and the physical compositions of the mud or other materials used in the construction of walls or thatch and their relation to residual toxicity of DDT.

In this inquiry attempts have been made to see that handling and treatment of insects both before and after exposure to the insecticide is kept at the minimum. An apparatus devised for testing residual toxicity has been described in the last year's report.

A method for spraying the test panels was devised. Dipping and the use of micro-pipette are too far removed from practice. Spraying

alone is the method of choice and in this inquiry attempts have been made to make the spraying of the test panels conform in a large measure to what is obtained in malaria control operations in the field, without at the same time losing sight of the required necessities for laboratory spraying, such as capacity to deliver equal doses repeatedly and an assurance that all parts of the test panels are fairly uniformly covered. The use of an ordinary Hudson pneumatic sprayer with a fan-shaped nozzle make the equipment very near to conditions of spraying.

In the spraying equipment devised for spraying the test panels, an ordinary Hudson pressure sprayer is used with a flat-spray nozzle. The test panels move on canvas belt running over two metal rollers driven by an electric motor. The insecticide is discharged at a pressure of 30-lb. to 28-lb. at which pressure the spraying of the panels is done and the pressure is not allowed to fall below 28 lb. The nozzle is adjusted so that the width of the spray just touches the edges of the panel and the entire surface is thus assured of a uniform spray.

METHOD OF WORKING.

- (1) The motor is switched on and the number of revolutions done by the canvas belt in a second is calculated.
- (2) The discharge rate of the pump per second is calculated at 30-lb. to 28-lb. pressure.
- (3) The time taken for the belt to travel a distance equal to the length of the panel is calculated.
- (4) The strength of the spraying fluid is adjusted to see that the required dosage is sprayed over the panel.

In this inquiry panels had to be sprayed in two dosages:—

- (i) 50 mg. per square foot.
- (ii) 200 mg. per square foot.

This meant that the panels which measured 6" × 5·7" have to be sprayed so as to get 11·8 mg. of DDT for the lower dosage or 47·2 mg. of DDT for the higher dosage.

The time taken for the panel to travel its own length was found to be 0·5 second.

In one second the sprayer, at 30-lb. to 28-lb. pressure, discharged 20 c.c. Therefore, in 0·5 second, the total discharge will be 10 c.c.

Now, this 10 c.c. must be made to contain the 11·8 mg. or 47·27 mg. of DDT as the case may, be so that the DDT may be uniformly sprayed over the test panel. In this strength alone, 5,000 c.c. have to be prepared so that the pneumatic sprayer may work satisfactorily.

A large zinc tray to contain the pneumatic sprayer and the moving belt was found useful in collecting the spilt DDT emulsion or suspension at the time of the spray.

TEST INSECTS.

Test insects were being reared under comparative conditions with the same quantity of food supply and water. The pupæ that develop on any one day have been utilized for the test. *A. stephensi* type has been used for all the tests recorded in this inquiry.

STUDY OF SOIL TYPE.

The Madras State is a part of the land surface of Indian peninsula. which has remained stable and undisturbed for untold geological ages. The parent rock of the geological formation is responsible for the variable soils present, as for instance the difference between the black and red soils or if they are of alluvial origin on the rock formation in the catchment area of the river bringing them down and depositing them in the deltas.

The soil.—The soil as ordinarily conceived consists of the following :—

- (a) Mineral materials.
- (b) Organic matter.
- (c) Water.
- (d) Air.

These, in the main, are in a fine state of subdivision and are intimately mixed. In fact, the contact is often so close as to render satisfactory separation rather difficult.

The mineral material has its genesis in the regolith or soil material. Some of the soil minerals have persisted more or less unchanged, while others have developed as the regolith weathered either before or during soil formation. Naturally, various sizes of particles occur ranging from those which are coarse, such as gravel and sand, to those, such as silt and clay which are in a fine state of division.

The organic matter represent the accumulation of plant and animal residues and is generally in an active state of decay.

An examination of any soil will reveal the presence of pore spaces of varying sizes. These occur not only between the large solid particles, but also between and within the clumps and aggregates which the fine particles tend to form. The pore spaces variable as to continuity, dimensions and total volume are occupied in large part by water and air, the proportion depending on the character of the soil and the conditions under which it is functioning.

Water, the third component of the soil is held within these pore spaces with varying tenacity due to certain surface forces. Soil water also carries many soluble salts.

The principal gases of the soil air, the fourth component, are nitrogen, oxygen and carbon dioxide.

Volume and composition.—A silt-loam surface soil contains approximately 50 per cent of solids and 50 per cent of pore space. This pore space will be somewhat less for sandy soils and somewhat more for soils of a more clayey nature. The 50 per cent of solid space is occupied by about 45 per cent of mineral and 5 per cent of organic substances by volume. The proportion of air and water is, of course, subject to great fluctuation under natural conditions depending on the weather and other factors.

In short, the soil may be visualized as possessing a frame work of mineral matter—in part non-colloidal and inert ; in part colloidal and extremely active . In mineral soils clayey materials make up much of this latter friction, but mixed with this viscous, galatinous mineral matter and

partially adsorbed thereby, is a certain amount of humus, notably colloidal and unstable clay and humus, more or less intimately mixed constitute most of the colloidal matter found in soils. This finely divided fraction not only promotes activity catalytically, but may also participate intensively in the changes that occur. In fact the latter may react and be reacted upon to the extent of losing its colloidal character in the production of simple compounds. Adsorption, plasticity, cohesion and certain chemical changes are all controlled by substances in the colloidal state. While humic colloids are the more active, the presence usually of a larger amount of viscous, gelatinous and more stable clayey matter preserves a balance in their relative importance.

In Agriculture five soil types referred to below are recognized in the Madras State :—

1. The red soils.
2. The black soils.
3. Coastal alluvium.
4. Delta soils.
5. Laterite soils.

Types 1, 3 and 5 are predominantly red, reddish or brown in colour, while types 2 and 4 are dark coloured popularly described as black. Type 1 occurs over a large part of the Madras State and may be said to be the common type. It occurs in all sorts of situations ranging from hill slopes to deep valleys between hills in various parts of the State. Types 3 and 5 are also red or reddish brown, though they form different soil types from the point of view of the soil chemist.

Type 2 is a characteristic soil occupying, for the most part, the central plateau of the peninsula.

Type 4, the delta soil, occurs mainly on the deltas of the rivers.

The laterite type 5 occurs in the West Coast and in some parts of the East Coast.

To start with, samples were obtained from six areas of the Madras State and the chemical analysis of the soils is furnished in Table I :—

TABLE I.
Chemical analysis of samples of earth.

Heads of analysis.	Araku, per cent.	Chaga- nur, per cent.	Ennore, per cent.	Dhim- bam, per cent.	Mala- bar, per cent.	Tonjore per cent.
<i>(a) Chemical analysis :</i>						
Moisture	0·30	6·02	3·01	4·36	2·82	0·82
Less in ignition	23·95	6·53	1·81	11·61	4·43	3·05
Iron oxide (Fe ₂ O ₃)	23·84	3·41	1·96	6·17	4·60	2·18
Alumina (Al ₂ O ₃)	46·73	7·60	3·95	12·87	8·71	7·43
Insolubles	5·6	8·5	91·14	68·74	81·98	87·08
<i>(b) Mechanical analysis :</i>						
Clay	10·53	47·13	19·45	38·63	21·35	27·33
Silt	8·88	14·10	1·10	11·55	9·22	2·57
Fine sand	8·92	18·06	3·06	14·01	21·06	15·24
Coarse sand	68·92	15·86	75·60	33·48	48·34	56·61
Acid solubles	2·75	4·81	0·79	2·33	0·03	...
TOTALS	100·00	100·00	100·00	100·00	100·00	101·75

It will be seen that the Araku soil was poor in moisture content, had a high percentage of Al_2O_3 and Fe_2O_3 among the six specimens of soil samples examined. As regards the physical composition, Araku had the lowest per cent of clay and highest percentage of coarse sand, while the sample from Bellary had the highest percentage of clay and silt, lowest of coarse sand. Judged by the chemical and mechanical analysis reports the Araku and Bellary chaganur soils seem to represent more or less extreme conditions among the six soil samples selected for the study.

OBSERVATIONS IN THE FIELD.

(i) Entomological observations.

Six areas were selected for field observations.

Area I. Araku valley (Vishakapatnam district) :

Six dwellings in a block were selected in Korrai, a village off the main road from Araku to Madakund, about five miles east of Araku village. The houses are all thatched with grass and the walls are all finished with a type of red coating derived from red ochre available in this area. Two houses in an adjoining block were kept as 'controls'. Another village, Kollaputti, in the Valley was selected for the second series of observations as the first village had to be included in routine anti-malarial operations.

The results of entomological observations made in this area are given in Table II :—

TABLE II.
Adult anopheline collection in Araku.

Date	Control				Suspension D				Emulsion E				Solution S			
	A		F		A		F		A		F		A		F	
	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.
1952	<i>I Series—Before DDT spraying.</i>															
24-11	...	13	...	5	...	37	...	25	...	15	...	3	...	16	...	6
	<i>After DDT spraying.</i>															
1-12	2	18	2	13
9-12	3	25	...	1
15-12	...	16	...	3
23-12	...	9	...	4
29-12	...	15	...	3
1953	<i>II Series—Before DDT spraying.</i>															
5-1	1	15	...	4	1	4	...	1
12-1	...	11	...	2	...	3	1	2
1953	<i>After DDT spraying.</i>															
4-4	...	25	...	9	...	31	...	11	...	36	...	13	...	23	...	5
4-4	...	7	...	2	...	3	3	...	1	...	3
19-4	...	4	...	1	2
3-5	...	14	...	3	1
19-5	...	17	...	4	3
3-6	...	7	...	3	...	5	13	...	3
18-6	...	14	...	3	No collection				...	7	...	1
10-7	...	22	...	1	...	15	...	1	...	27	...	1	...	15	...	2
3-8	...	17	...	3	...	16	13	...	1	...	17	...	3
23-8	...	11	...	1	...	9	...	1	...	7	...	1	...	11	...	1

A = *Anopheles* all species.

F = *Anopheles fluviatilis*.

M = Male,

T = Total.

For four weeks no anopheline prevalence was observed in the sprayed houses and for six weeks no *A. fluviatilis* could be captured in the sprayed huts in the first series of observations, although *A. fluviatilis* adults were caught in the 'control' group of huts. In the second series of observations *A. fluviatilis* was captured on the afternoon of the spray day and then nine weeks after the spray.

Area II. Chaganur (Bellary district) :

Six huts were selected, three Harijan quarters and three others, in Chaganur (Bellary district) on the banks of Hagari and about ten miles from Bellary. The latter were combined human dwelling and cattlesheds. The houses in this area have generally high walls without outside windows. Cattle usually occupy the front portion. The walls are generally of rough stone in mud and the roof is nearly flat. Faggots and a foot or so of mud are placed on the rafters and coated outside with clay. Some parts of the wall are smeared with red earth and some parts with lime wash and still others with cowdung.

The results of the observations are indicated in Table III :—

TABLE III.

Adult anopheline collection in Chaganur.

Date	HARIJAN QUARTERS Control				Suspension D				OTHERS Emulsion E				Solution S			
	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.
<i>Before DDT spraying.</i>																
29-11-52	33	56	14	27	6	9	...	2	8	38	...	6	8	18	...	2
<i>After DDT spraying.</i>																
5-12-52	...	15	...	3	9	...	1	...	2	...	1
12-12-52	...	5	5	...	4	...	6	...	1
16-1-53	...	1	3	2
30-1-53	...	14	12	...	1
6-2-53	...	7	...	3	...	1	7	...	2	1
16-2-53	...	2	2	2	...	1	...	1
23-2-53	...	3	...	1	4	...	1
2-3-53	...	1	...	1	...	1	2
9-3-53	...	3	3	1
3-4-53
20-4-53	...	2	1	1
5-5-53
20-5-53

M=Male.

T=Total *Anopheles*.

For eight weeks no anophelines were collected in the Harijan quarters, though mosquitoes were captured from the 'control' houses.

An analysis of the figures furnished in Table III is given in Table IV:—

TABLE IV.

Adult anopheline collection in different types of walls in Chagánur.

Date of collection	Control		Suspension		Emulsion		Solution	
	M.	T.	M.	T.	M.	T.	M.	T.
(1) <i>Cow-dung-coated walls.</i>								
<i>Before DDT spraying.</i>								
28-11-52	15	...	31	...	3
<i>After DDT spraying.</i>								
5-12-52
12-12-52
16- 1-53
30- 1-53
6- 2-53
16- 2-53	...	3
(2) <i>Lime-washed walls.</i>								
<i>Before DDT spraying.</i>								
28-11-52	...	6	5	7	1	17	...	6
<i>After DDT spraying.</i>								
5-12-52	1	...	2	...	1
12-12-52	1	...	4	...	1
16- 1-53	2
30- 1-53	...	6
6- 2-53	...	5	...	1	...	2
16- 2-53	1	...	1	...	1
(3) <i>Red-earth-coated walls.</i>								
<i>Before DDT spraying.</i>								
28-11-52	...	1	...	3	...	1	...	3
<i>After DDT spraying.</i>								
5-12-52	...	4	...	2	...	1	...	1
12-12-52	...	2	...	3	...	2
16- 1-53
30- 1-53	...	4	...	1
6- 2-53	2	1
16- 2-53	1
23- 2-53	1

Only 28 per cent (29 out of 104) were captured from walls, the remaining large per cent was captured for cobwebs, clothes and hangings.

No mosquito was anywhere captured from the dung-smeared sprayed walls or from plain clay area, although mosquitoes were captured from the red-earth and lime-coated areas.

Area III. Eranavur (Chingleput district) :

Six huts were selected in Eranavur, nine miles north of Madras on the Madras Ennore road. Three of the houses were mud walled and lime washed and three others were cowdung coated. All the houses were thatched with palmyra leaves. Four more huts, two white washed and two cowdung coated, were kept as 'controls'. Two series of observations were made as shown in Tables V and VI :—

TABLE V.

Adult anopheline collection in lime-washed walls in Eranavur.

Date	Control				Suspension				Emulsion				Solution			
	A.		C.		A.		C.		A.		C.		A.		C.	
	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.
<i>Series I.</i>																
<i>Before DDT spraying.</i>																
1952 14-11	41	93	1	2	25	69	2	2	17	53	...	6	12	59
<i>After DDT spraying.</i>																
20-11	9	56	4	...	1	...	3	...	1	...	10
27-11	12	76	...	3	4	9	2	6	6	18
4-12	4	43	3	19	1	9	Not collected			
18-12	21	60	8	...	1	...	11	...	1	18	63	...	1
26-12	6	19	4	3	1	8
<i>Series II.</i>																
<i>Before DDT spraying.</i>																
1953 16-1	8	18	9	15	10	20	...	1	6	23
<i>After DDT spraying.</i>																
28-1	5	10	2	...	1	...	2	...	1
5-2	1	4	1	2	1	...	1
13-2	3	9	2	3	...	3	1	3	2	...	1
21-2	11	22	2	3	...	1	Not collected				1	2	1	2
26-2	16	34	6	8	...	2	2	5	...	2
13-3	2	11	1	2	2	5	...	1	1	2	...	1
20-3	4	9	1	3	4	1	1
27-3	...	2	Not collected				...	1
10-4	4	13	2	4	7
24-4	2	10	1	4	1	3	2	8	1	5

A=*Anopheles* all species.

C=*Anopheles culicifacies*.

M=Male.

T=Total.

TABLE VI.

Adult anopheline collection in cowdung-smeared walls in Eranavur.

Date	Control				Suspension				Emulsion				Solution			
	A.		C.		A.		C.		A.		C.		A.		C.	
	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.
<i>Series I.</i>																
<i>Before DDT spraying.</i>																
1952 14-11	44	155	...	1	32	84	27	104	...	2	4	41
<i>After DDT spraying.</i>																
20-11	35	88	...	7
27-11	32	96	...	3	...	3	4	18
4-12	9	37	...	1	5	12
18-12	27	69	1	8
26-12	13	24	1	1
1953 2-1	10	34	...	3
8-1	11	33	...	3
<i>Series II.</i>																
<i>Before DDT spraying.</i>																
1953 16-1	15	36	13	23	43	74	14	22
<i>After DDT spraying.</i>																
28-1	2	8	1	1	1	1	1	1
5-2	4	11	...	2
13-2	6	25	...	2
21-2	9	28	1	2	Not collected				...	1	...	1	...	2
26-2	10	51	3	4	3	...	3	1	5	1	2
13-3	9	23	3	3	5	8	...	1
20-3	8	21	...	1	Not collected				...	2	4	12
27-3	2	9	...	1	"				...	3	...	1	2	5
10-4	6	17	2	3	"				1	7
24-4	3	8	1	3	"				...	1	2	6	1	1

A=*Anopheles all species.*C=*Anopheles culicifacies.*

M=Male.

T=Total.

The anopheline prevalence is comparatively very low in the houses with cowdung-coated walls.

Area IV. Dhimbam (Coimbatore district) :

This area is situated on the hills and as most of the areas had already received DDT spray this area was not found suitable for field entomological observations. An unsprayed group of huts in the middle of the sprayed area was, however, selected and sprayed, one house with one type of formulation. No mosquitoes could, however, be captured in the area both before or after spraying.

Area V(a). Uppinangadi, (South Kanara):

Three houses with walls made of laterite blocks of the usual type in this area were selected and sprayed each with one type of formulation, leaving one as 'control'. The thatch is made of coco-nut leaves. The results of the entomological observations made in this area are furnished in Table VII :—

TABLE VII.

Adult anopheline collection in Uppinangadi. (Laterite area).

Date	Control				Suspension				Emulsion				Solution			
	A.		C.		A.		C.		A.		C.		A.		C.	
	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.
<i>Before DDT spraying.</i>																
8-2-53	...	5	...	3	...	2	2	...	1
<i>After DDT spraying.</i>																
9-2-53	...	15
25-2-53	1	11	1	3
10-3-53	3	19	3	11	...	1	1
25-3-53	2	9	2	5	1	...	1
9-4-53	...	7	...	5	...	1
21-4-53	1	4	1	2	...	1	2
10-5-53	1	2	1	2	...	1	1
3-6-53	4	16	4	9	...	2	...	2	2	7	2	6	1	3	1	3
18-6-53	...	7	...	2	1
1-7-53	...	3
19-7-53	...	4	1
7-8-53	...	1

A=*Anopheles all species.*

C=*Anopheles culicifacies.*

M=Male.

T=Total.

For four weeks after the spray, no mosquito could be captured, although an appreciable number of mosquitoes could be captured from the control huts.

Area V(b). Muthalamada, (Malabar district):

Two groups of huts were sprayed in Muthalamada amson of Malabar district. Of the two groups sprayed, in one (Santhamaniyakkara Kalam) all the walls and roofs are made up of palmyra leaf only, while in the other group (Nagappanaicken Kalam) the walls are made up of mud. DDT was sprayed in three formulations, one hut in each of the groups mentioned above receiving one type of formulation.

The results of entomological collections are given in Table VIII :—

TABLE VIII.

Adult anopheline collection in Muthalamada (Laterite area).

Date	Control				Suspension				Emulsion				Solution			
	A.		F.		A.		F.		A.		F.		A.		F.	
	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.
1953	(1) <i>Santhamaniakkara Kalam.</i> <i>Before DDT spraying.</i>															
11-7	1	4	1	2	1	2	2	5	1	2	2	4	...	2	...	5
	<i>After DDT spraying.</i>															
3-8	...	8	...	6
22-8
13-9	1	10
1953	(2) <i>Nagappanaicken Kalam.</i> <i>Before DDT spraying.</i>															
11-7	3	12	3	10	3	13
	<i>After DDT spraying.</i>															
3-8	3	19	1	5	...	1	1
22-8
13-9	...	4	3

DDT is effective in all types of formulations for eight weeks in Santhamaniakkara Kalam with human dwellings—all made up of palmyra leaves, while comparatively speaking, it does not seem to be so effective in the other group with mud-walled houses.

Area VI. Pattukottai, (Tanjore district):

Six huts in Latchathope, about one mile from Pattukottai, were sprayed, two huts receiving one type of formulation. The huts are all mud-walled with lime wash and thatched with palmyra leaves. The results of observations made in the area are shown in Table IX :—

TABLE IX.

Adult anopheline collection in Pattukottai.

Date	Control				Suspension				Emulsion				Solution			
	A.		C.		A.		C.		A.		C.		A.		C.	
	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.	M.	T.
	<i>Before DDT spraying.</i>															
5-11-52	8	140	1	2	71	128	2	5	56	94	57	83
	<i>After DDT spraying.</i>															
6-11-52	58	98	1	1	10	14	3	14	3	16
13-11-52	35	82	3	14	11	28	2	8
20-11-52	10	28	...	1	1	9	3	18	...	1	1	5
28-11-52	14	25	...	2	2	6	5	8	...	1	1	5

A rapid fall in the adult mosquito prevalence is observed in all the sprayed huts. As the mosquitoes are captured, mostly from the thatch, the results do not furnish an index of the efficiency of residual toxicity of the DDT sprayed on the walls.

(ii) *Chemical observations.*

Wall scrapings for Alessandrini test for the presence of DDT residue were collected from all areas. The samples were taken mostly from five situations two from exposed areas, one at about 5 ft. and the other at 2 ft. from the floor. These were usually taken from the walls opposite the door which is most exposed to sunlight in an ordinary human dwelling in the malarious areas. Two other samples at the same level from the floor were taken more inside and less exposed to light. A sample of bamboo or other thatch, or ceiling, also is included. The results of examination are furnished in Table X :—

TABLE X.
Chemical estimation of DDT by Alessandrini method.

Formulation	Number of scrapings examined	Exposed number positive, per cent	Unexposed number positive, per cent
		<i>Araku.</i>	
D	30	7	8
E	30	5	5
S	30	5	7
		<i>Ghaganur.</i>	
D	14	1	4
E	10	5	1
S	12	...	1
		<i>Eranavur.</i>	
D	48	5	5
E	48	4	4
S	48	2	4
		<i>Dhimbam.</i>	
D	30	1	1
E	30	4	3
S	30
		<i>Uppinangadi.</i>	
D	51	18	23
E	57	23	24
S	55	42	19
		<i>Pattukottai.</i>	
D	44	11	4
E	47	8	2
S	46	4	2
TOTALS ...	660	147 (22·6)	117 (17·7)

D=Suspension.

E=Emulsion.

S=Solution.

There was no difference in the per cent of samples positive between scrapings taken from exposed and unexposed situations.

The scrapings positive by weeks after the spray are furnished in Table XI :—

TABLE XI.

Weeks after spray		Suspension				Emulsion				Solution			
		1	2	3	4	1	2	3	4	1	2	3	4
I. <i>Araku.</i>													
2 hours	...	8	3	38	20	8	4	50	4	8	0	0	0.0
25 weeks	...	8	5	63	9	8	4	50	12	8	4	50	4.0
II. <i>Eranavur.</i>													
2 hours	...	12	4	33	56	12	4	33	56	15	5	31	20
6 weeks	...	12	1	83	40	12	...	0	0.0	16	0	0	0.0
III. <i>Uppinangadi.</i>													
2 hours	...	5	4	80	36	7	6	81	28	5	3	60	24
3 weeks	...	5	4	80	36	5	5	100	28	5	2	40	24
7 weeks	...	5	3	60	24	5	4	80	24	5	2	40	28
9 weeks	...	6	4	80	28	5	4	80	32	5	4	80	16
2 weeks	...	5	4	80	4	5	4	80	4	5	4	80	4

1=Number of scrapings examined, 2=Number found positive, 3=Per cent positive.
4=Average DDT contents in mg. per sq. ft.

In Araku, the scrapings were not positive in areas sprayed with DDT solution even two hours after spraying. In Eranavur six weeks after the spray, only scrapings from walls sprayed with DDT suspension were positive. In the laterite West Coast DDT is positive in the wall scrapings taken for over three months, but the average DDT content per sample is reduced after the ninth week.

OBSERVATIONS IN THE LABORATORY.

The field observations given in detail above were the basis for the laboratory observations reported below. Kruse and Konchady (1950) found that cowdung-carbon-surfaced panels appeared to have good residual effect for over six weeks.

Earth obtained from six different areas was filled in different trays and different panels were thus prepared. Over each type of earth, two kinds of surface treatment (cowdung and lime-wash), were given and one panel was left with plain earth having no special coating. The other panels prepared included bamboo which was distinguished into three types : (i) With only the hard smooth outer surface of bamboo exposed, (ii) with the spot inner surface of the split bamboo exposed and (iii) with bamboo matting woven with thin tape-like strips shaved off from bamboo, leaves of coco-nut and palmyra palms and straw, the latter three are mostly used as thatching materials, though some times they form part of the walls of human dwellings.

Only emulsions and suspensions were the formulations tried. The dosages employed were 50 mg. and 200 mg. per sq. ft.

The panels were carefully prepared and dried in the shade and sprayed with the two dosages referred to above. The panels were applied to the test insects unusually late in the afternoons. The time of exposure to the sprayed surface was 30 minutes. Mortality rates were calculated as per Fay's formula. One set of 'control' was run on every day of the experiment. In these 'controls' mosquitoes were exposed to an unsprayed panel and after 30 minutes of such exposure, the plunger was raised and the mosquito mortality observed during the next 24 hours. Excepting eleven instances out of 54 such 'controls', there was no mortality during the first 24 hours and even in these eleven instances, the mortality was less than ten per cent.

Mean mortality rate for eight weeks is an average of the rates obtained during every week of the experiment. Although it may not be statistically correct, it is still used as it gives a convenient index for comparative purposes.

Formulation I-DDT-Aromex emulsion.

Dosage (a) : 50 mg. per square foot.

TABLE XII.
DDT-residual toxicity with plain earth samples.

Earth sample	Twenty-four hours mortality in weeks after spray								Mean mortality 8 weeks
	1	2	3	4	5	6	7	8	
1. Araku ...	100	62	67	50	30	23	22	29	48
2. Chaganur ...	55	69	67	70	72	63	62	60	65
3. Eranavur ...	100	95	83	67	75	37	23	24	63
4. Dhimbam ...	85	62	39	45	27	24	...	29	39
5. Muthalamada ...	73	53	...	45	40	51	54	45	52
6. Pattukottai ...	70	50	60	67	33	40	...	33	50

Chaganur shows the highest mean mortality.

Fifty per cent mortality is recorded up to second week in Dhimbam, fourth week in Araku and Pattukottai.

TABLE XIII.
DDT-residual toxicity with cowdung-smeared earth samples.

Earth sample	Twenty-four hours mortality in weeks after spray								Mean mortality 8 weeks
	1	2	3	4	5	6	7	8	
1.	94	100	100	100	91	57	61	41	69
2.	74	52	68	70	68	67	57	61	65
3.	40	95	100	...	57	61	54	...	68
4.	96	91	73	69	45	52	49	52	66
5.	68	71	67	53	62	49	60	39	59
6.	47	70	100	71	41	...	39	27	58

Dhimbam and Araku show better results than plain earth samples.

Fifty per cent mortality is recorded up to the sixth week in Araku, eight weeks in Chaganur and Dhimbam.

TABLE XIV.

DDT-residual toxicity with lime-washed earth samples.

Earth sample	Twenty-four hours mortality in weeks after spray								Mean mortality 8 weeks
	1	2	3	4	5	6	7	8	
2.	53	69	...	75	69	61	59	39	61
3.	67	100	100	52	47	49	35	48	62
4.	56	71	...	59	47	62	45	33	53
5.	82	84	...	61	52	41	34	29	55
6.	56	56	95	...	59	44	...	41	59

Fifty per cent mortality has been recorded up to seventh week with Chaganur sixth week with Dhimbam, fifth week with Muthalamada and Puttukottai, and fourth week with Eranavur lime-washed samples.

TABLE XV.

Residual toxicity with thatching materials.

Sample	Twenty-four hours mortality in weeks after spray								Mean mortality 8 weeks
	1	2	3	4	5	6	7	8	
Bamboo (inner)	83	25	32	27	42	...	23	22	36
Bamboo (outer)	83	50	45	65	37	...	41	47	53
Bamboo (mat)	100	100	90	82	73	69	86
Palmyra leaf	92	51	47	51	49	43	56
Coco-nut leaf	79	94	79	67	43	25	...	7	56
Straw	100	30	19	19	...	17	37

With one exception of bamboo matting, 50 per cent mortality has been observed not beyond the fourth week and with straw and bamboo (inner) not beyond the first week. Highest mean mortality is recorded with bamboo mat and next leaves of palmyra and coco-nut palm. The bamboo with the hard outer surface comes next.

Note :—The serial numbers of earth samples in Tables XIII, XIV, XVI, XVIII, XIX, XX and XXII relate to the same earth samples as in Table XII.

Dosage (b) 200 mg. per square foot.

TABLE XVI.

Residual toxicity with plain earth samples.

Earth sample	Twenty-four hours mortality in weeks after spray								Mean mortality 8 weeks
	1	2	3	4	5	6	7	8	
1.	91	81	50	35	23	20	15	20	42
2.	79	80	53	45	27	...	31	...	53
3.	85	89	70	54	47	...	53	42	63
4.	71	53	30	25	31	40	...	32	40
5.	83	52	...	43	37	...	27	23	44
6.	100	81	...	62	62	...	47	32	64

No special advantage is seen with the higher dosage.

TABLE XVII.

Residual toxicity with thatching materials.

Sample	Twenty-four hours mortality in weeks after spray								Mean mortality 8 weeks
	1	2	3	4	5	6	7	8	
Bamboo (inner)	100	89	...	67	...	33	...	33	64
„ (outer)	100	75	...	67	...	35	...	67	69
„ (mat)	100	100	...	75	...	75	...	67	83
Palmyra leaf	100	100	...	100	...	50	...	33	78
Coco-nut leaf	100	100	...	100	...	67	...	50	83
Straw	100	09	...	100	...	67	...	60	85

Over 50 per cent mortality is maintained for eight weeks with bamboo (outer, inner, mat) and straw. Mean mortality rates are much higher than those obtained with samples of earth.

Formulation II—suspension.

Dosage (a) : 50 mg. per square foot.

TABLE XVIII.

Residual toxicity with plain earth samples.

Earth sample	Twenty-four hours mortality in weeks after spray								Mean mortality 8 weeks
	1	2	3	4	5	6	7	8	
1.	90	90	100	50	60	33	33	25	59
2.	95	100	90	67	60	67	40	25	67
3.	100	100	100	91	73	75	67	67	84
4.	96	80	71	50	...	67	50	33	64
5.	100	100	67	50	67	67	33	25	64
6.	100	100	100	57	50	67	30	33	69

Fifty per cent mortality is over eight weeks with Eranavur and up to seventh week with Dhimbam and Pattukottai. With Araku sample 50 per cent is reached by the fifth week.

Increased mean mortalities have been observed over those recorded with emulsion of either dosage.

TABLE XIX.

Residual toxicity with cowdung-washed earth samples.

Earth sample	Twenty-four hours mortality in weeks after spray								Mean mortality 8 weeks
	1	2	3	4	5	6	7	8	
1.	100	100	...	50	67	50	73
2.	100	100	...	67	50	33	70
3.	100	90	...	67	67	25	69
4.	100	80	...	50	50	33	63
5.	100	75	...	80	50	50	71
6.	100	80	...	50	33	33	59

Increased mean mortality rates are observed in Araku, Chaganur and Muthalamada.

TABLE XX.

Residual toxicity with lime-washed earth samples.

Earth sample	Twenty-four hours mortality in weeks after spray					Mean mortality 8 weeks
	1	2	4	6	8	
2.	90	80	...	67	50	57
3.	67	80	...	50	...	66
4.	75	100	75	50	45	69
5.	75	90	50	53	...	62
6.	90	75	50	33	40	58

Fifty per cent mortality has been observed up to the eighth week with Chaganur and up to the sixth week with the other samples except Pattukottai where 50 per cent mortality was observed only up to the fourth week.

TABLE XXI.

Residual toxicity with thatching materials.

Sample	Twenty-four hours mortality in weeks after spray					Mean mortality 8 weeks
	1	2	4	6	8	
Bamboo (inner)	80	100	75	67	50	74
„ (outer)	100	100	100	80	67	89
„ (mat)	100	75	80	100	80	87
Palmyra leaf	100	80	100	80	80	88
Coco-nut leaf	100	80	75	80	80	83
Straw	100	100	94	80	80	91

Fifty per cent mortality has been observed with all types up to the eighth week. The mean mortality rates show increased effect over that recorded with emulsion.

Very high mortality rates have been observed up to the sixth week.

TABLE XXII.

(b) ; Dosage 200 mg. per square feet.

Earth sample	Twenty-four hours mortality in weeks after spray								Mean mortality 8 hours
	1	2	3	4	5	6	7	8	
1.	100	100	100	60	67	50	67	40	73
2.	100	90	75	80	80	80	50	30	73
3.	100	100	90	70	40	50	65	30	68
4.	100	90	100	...	60	75	50	23	71
5.	100	100	100	50	40	67	76
6.	100	80	80	...	80	55	56	67	74

Fifty per cent mortality is recorded up to eighth week with Muthalamada and Pattukottai samples and up to seven week with the others.

Mortality is higher with the higher dosage as compared with the lower dosage of the same formulation.

TABLE XXIII.

Residual toxicity with thatching materials.

Sample	Twenty-five hours mortality in weeks after spray					Mean mortality 8 weeks
	1	2	4	6	8	
Bamboo (inner)	80	100	45	75
„ (outer)	90	100	90	...	50	83
„ (mat)	100	100	100	...	40	85
Palmyra leaf	100	90	100	90	80	92
Coco-nut leaf	90	100	100	80	45	83
Straw	100	100	80	100	50	86

Fifty per cent is recorded up to the eighth week with bamboo (outer), palmyra leaf and straw. Very high mortality rates have been obtained up to the sixth week.

There is no significant increase in the mean mortality rates over those recorded with the lower dosages.

DISCUSSION.

The field observations indicated that the prevalence of mosquitoes as studied by routine mosquito collections was comparatively low in DDT-sprayed houses with cowdung-coated walls. Though it is very hard to say whether the decreased prevalence was due to increased mortality or due to increased excitation, laboratory tests on panels indicate the presence of increased toxicity for longer duration. This applies equally well to both emulsions and suspensions.

Chemical constituents of the earth do not seem to affect DDT-residual effect for the mortality rates obtained in the laboratory are generally over 50 per cent for nearly four weeks with all samples of earth, although the rates vary afterwards. Porosity and interspace between the particles seem to play a chief rôle in prolonging DDT-residual toxicity. From this aspect the six types of earth may be reduced to two chief types : (i) clayey non-porous Chagannur type, and (ii) sandy or porous Araku type. The mean mortality rates obtained over a period of eight weeks indicate that the sample of earth from Bellary has good residual toxicity with DDT emulsions when sprayed at 50 mg. per square foot and this index is not increased at the higher dosage of 200 mg. per square foot. With Araku type, the mean mortality rate over the same period is very low and the weekly mosquito mortality rate goes down after four weeks and the rates recorded after the fourth week are less than 30 per cent. The wall surface in human dwellings, however, seems to possess enough toxic effect to control *A. fluviatilis*, for this species could not be collected for nearly two months, although other species of anophelines have been collected within two weeks in a house sprayed with DDT solution in kerosene. This may be due to the special habits of *A. fluviatilis* and its low rate of prevalence. In Araku, the red coating which is usually given to the walls is derived from red ochre which is highly porous, easily absorbs the DDT sprayed as solution. The emulsions and to a still greater extent the suspensions do not easily penetrate inside and may be this is responsible for the better residual toxicity obtained with these two types of formulations. Chemical tests of wall scrapings by Alessandrini method immediately after the DDT spray seem to confirm this point. Laboratory tests with panels of Araku earth smeared with cowdung and sprayed with DDT emulsion indicate an increase in the duration of its residual toxicity. This cannot, however, obviously be a suggestion for adoption for the red ochre gives an effect to the interior of the house which dwellers in such types of houses can rarely be persuaded to change to cowdung wash.

As regards the other surfaces, the same physical condition of porosity seem to affect the residual toxicity of DDT. The bamboo (matting) made of bamboo shavings is more absorbent than the outer hard surface of the bamboo. The bamboo (inner surface) is porous and part of the sprayed

insecticide goes inside. It, however, seems from the laboratory tests that the bamboo, although absorbent, is still able to give enough lethal dose to the insect. This is perhaps due to the limited absorbing capacity of the thin bamboo shavings. The coco-nut leaf seem to be comparatively more porous than the palmyra or the straw. Further, the type of crystal formation on these surfaces may affect residual toxicity. In the case of palmyra leaf, the crystals are mostly flat, although a few project out. In bamboo matting the crystals tend to project although they seem in general to be sparser than in the other thatching materials studied. The crystals formed on the palmyra leaf are longer than that formed on the straw. This comparison in the size, etc., could be made because all the surfaces were combined in one panel, sprayed and observed. Probably the projecting types are the crystals which decide mosquito mortality, as they are likely to be easily taken by the insect. The comparatively better results obtained in Santhamaniyakkara Kalam in Muthalamada may perhaps be due to this factor.

The strength of the formulation used over such structures, such as the palmyra leaf, has to be seriously considered in practical mosquito-control operations. Further, efforts should be made to spray the thatch from different angles so that no part of the surface is left unsprayed.

From what is discussed above, it may perhaps be stated that a non-porous background, such as a good clayey earth may prevent letting in the insecticide sprayed either as suspension or emulsion and further such a non-porous background may enable the insecticide particles either as crystals or supersaturated droplets to settle mostly on the surface. The clayey portion may have to be suitably reduced in practice as otherwise clay by itself may tend to crack very much on drying. On this background, lime wash may be given and, after sometime, sprayed with DDT. In those places where cowdung wash is in vogue that practice may be encouraged from mosquito-control point, though not from any other aspect. It is likely that an initial filling up of the space between the particles with same non-porous material may be of distinct advantage in lengthening the duration of residual toxicity. This perhaps is the action in the case of cowdung and perhaps this may be equally well done by clay or any other non-porous material, which would increase the duration of residual toxicity of DDT.

The results of Alessandrini tests done show that there is no practical difference in DDT content between dark and illuminated portions of the inside of houses where the scrapings have been taken. The illumination obtained in the interior of most of the human dwellings in malarious areas which portions alone are sprayed in indoor DDT-residual spraying campaign, does not seem to be adverse to DDT-residual toxicity.

Finally, there seems to be no special advantage in spraying at the high dosage of 200 mg. per square foot either as emulsion or suspensions over that obtained with a dosage of 50 mg. per square foot in the limited observations made during this inquiry.

SUMMARY AND CONCLUSIONS.

1. New types of equipments for testing residual toxicity and for spraying the test panels are described.

2. The field observations made in different areas and the laboratory observations are both given in detail.

3. The practice of smearing the walls with cowdung prior to DDT spray seemed to increase the residual toxicity of DDT.

4. Porosity and size of inter-particular space of the material with which walls are covered seem to be important factors that affect residual toxicity of DDT.

5. The black-cotton clayey-coil sprayed with DDT has high mean mortality for eight weeks.

6. A non-porous background to the spraying surface is suggested and a thorough spraying of the lower layers of the palmyra or coco-nut leaves which form the roof of human dwellings in malarious areas is advocated.

7. The outer hard surface of the bamboo is preferable to the inside as regards residual toxicity of DDT. The thin shaving of bamboo used for making bamboo mats is also equally good as the outer surface.

8. DDT-residual toxicity is not adversely affected by spraying over lime-washed walls.

9. There seems to be no special advantage in spraying at the high dosage of 200 mg. per square foot either as emulsion or suspension in the surfaces tested.

5. Inquiry for making detailed study of the malaria parasites in the Himalayan flying squirrel (*Petaurista inornatus*) and the Himalayan Langur (*Semnopithecus schistaceus*) under Dr. H. N. Ray at the Indian Veterinary Research Institute, Mukteswar-Kumaon, U.P.

During the current year several Langurs were shot on the dates noted against each.

Month of June 1953.

One Langur killed on	4-6-53	...	Negative
Two Langurs killed on	6-6-53	...	Negative
One Langur killed on	8-6-53	...	Positive
One „ „ „	26-6-53	...	Positive

From the positive Langur which was killed on 8-6-1953, 12 malarial cysts were collected from the liver. These were emulsified in 2 c.c. of Ringer's solution, filtered through a fine muslin to avoid coarse particles, and inoculated into two experimental monkeys (*Macacus*) (Nos. 2 and 3), giving 1 c.c. of the emulsion to each.

Monkey No. 2 which was infected on 8-6-53 showed a rise of body temperature on the 9-6-53 (103°F.) in the morning, and (103.6°F.) in the evening. While monkey No. 3 which was also given 1 c.c. of the emulsion

on the same day, showed a rise of body temperature on 10-6-53 (102.6°F.) in the morning, and (103.2°F.) in the evening.

From the positive Langur killed on 26-6-53, 22 malarial cysts were collected again from the liver, and these were emulsified in 4 c.c. of Ringer's solution. The same two monkeys (Nos. 2 and 3) were re-infected with a heavier dose (2 c.c. each of the emulsion), and a regular check on the temperatures kept.

Although the monkeys showed a rise in temperature from time to time, they have so far failed to show any sign of infection, on daily examinations of bloodsmears.

Month of July 1953.

Two Langurs were killed on 27-7-53 ... Negative

One Langur was killed on 30-7-53 ... Negative

Month of August 1953.

One Langur was killed on 4-8-53 ... Negative

One " " " 11-8-53 Negative

One " " " 27-8-53 ... Positive

From the positive Langur which was killed on 27-8-53, nine malarial cysts were collected from the liver. These were emulsified in 1 c.c. of citrate solution, filtered and inoculated to experimental monkey No. 1 on 27-8-53.

The monkey showed a rise of body temperature on 29-8-53 (103.6°F.).

Although a daily check on the temperature and blood-smears are made, the smears have so far failed to reveal any infection. However, it was noticed that the monkey seemed to be very dull and inactive. It was also off its normal feed on three occasion. At the time of writing this report all the monkeys are negative for malaria parasites.

Laboratory animals, rabbits, guinea-pigs, rats and mice have so far remained refractory to infection with this malaria parasite from the Langur.

In August 1953, a number of mosquito larvæ was collected by the Insect-Collector in a pool of rain-water at a height of 7,400 feet above sea level. Maximum and minimum temperatures recorded during the period of collection were 72°F. and 58°F. , respectively. Though an adequate insectorium is not available here, attempts are being made to raise a colony of these mosquitoes. Mosquitoes have been identified by the Malaria Institute of India, as follows :—

- (a) *Aedes* (Finlaya) *pulchri* Venter.
- (b) *Aedes* (Finlaya) *pseudotæniatus*.
- (c) *Culex* (Culiciomyia) *virid* Venter,

During the period under review the usefulness of phase-contrast microscopy in the study of malaria parasite in unfixed and unstained blood films was recognized. Particularly the nature of pigment and its distribution in the parasite could be observed with great clarity without any masking effect of the stain. Photo-micrographs of *P. semnopitheci*, *P. knowlesi*, *P. berghei* and *P. gallinaceum* will speak for itself about the value of such observations. Detailed observations are now being made to study the pattern of pigmentation in these parasites with the help of phase-contrast microscope.

The malaria parasite of the Himalayan Langur (*Semnopithecus schistaceus* Skuse), has been identified as *Plasmodium semnopitheci* Henowles, 1919. According to Garnham, however, the parasite will have to be designated as *Hepatocystis semnopitheci*. All attempts to find erythrocytic schizogony in this parasite has met with negative result.

Cysts in liver when examined in section were in no way different from these described as merocyst from various species of malaria parasites. Groups of small cysts of E E phase as seen in the liver of flying squirrel (Ray, 1949) were not seen in the liver of infected Langurs. Nuclei of merozoites in a nature merocyst were Feulgen positive.

During the year under report specimens of flying squirrels could not be procured for study.

6. Investigation of the possibility of controlling the incidence of kala-azar by spray-killing of adult sandflies under Dr. B. C. Basu at the School of Tropical Medicine, Calcutta.

SURVEY OF SANDFLIES AROUND CALCUTTA CITY.

As mentioned in previous year's report the field studies are being continued at Thakurpukur and adjacent villages but from the last week of May 1953 (i.e. after completion of one year's study at Thakurpukur) some of the insect collectors were shifted from Thakurpukur to conduct a monthly survey of *Phlebotomus* flies throughout the fringe areas of the Calcutta City with a view to study the seasonal incidence of sandflies around Calcutta City and to establish its correlation with that of kala-azar. The entire fringe area of the city up to Metiaburz and Shibpur in the South, Khardah and Serampur in the North, Tangra in the East and Santragachi in the West is fairly heavily infested with *Phlebotomus argentipes*, *P. minutus* and *P. papatasi* have also been recorded in some of the areas.

BREEDING SITES OF SANDFLIES.

Search for natural breeding sites of sandflies is being continued, particularly in the houses maintaining poultry, cattle and other domesticated animals. Good collection of adults could be obtained from such sites but a very few larvæ could be collected.

Studies on incidence, sex ratio, blood meal, etc., of sandflies.

Month	Incidence	Male	Female	Fed	Unfed	Gravid
October 1952 ...	1,843	352	1,491	938	263	290
November 1952	831	231	600	475	63	62
December 1952	647	76	571	383	106	82
January 1953 ...	619	95	524	337	94	93
February 1953 ...	328	53	275	170	47	58
March 1953
April 1953 ...	815	98	717	439	138	140
May 1953 ...	1,663	183	1,480	920	257	303
June 1953 ...	163	22	141	98	19	24
July 1953 ...	985	158	827	471	133	223
August 1953 ...	1,260	175	1,085	749	114	222
September 1953	1,122	230	892	617	82	194

EFFECT OF SEASON ON THE LIFE-HISTORY OF SANDFLIES.

In the cold months namely, December, January and February (in Calcutta), the eggs did not hatch and in other months they hatched and the period for completing the life-cycle varied from 20 days to 52 days in different months.

Month	Number of days taken to complete life-history	Month	Number of days taken to complete life-history
January ...	Egg did not hatch	July ...	23
February ...	—Do.—	August ...	31
March ...	X	September ...	20 & 24
April ...	22	October ...	X
May ...	27	November ...	Eggs hatched and took 52 days.
June ...	29	December ...	Eggs did not hatch

LONGIVITY OF SANDFLIES.

Laboratory-bred *P. argentipes*, fed on raisins for 24 hours, were re-fed on cases of K. A. and D. L. and were kept at room temperature in chimneys with sufficient quantity of water but in no case they survived for more than two days in room-temperature conditions.

EFFECT OF INSECTICIDES ON DIFFERENT PHASES OF SANDFLIES.

The effect of pyrethrum extract two per cent (1 in 20), BHC (0.1 per cent) and DDT (five per cent) on egg, larva, pupa and adult sandflies (*P. argentipes*) has been tested under laboratory conditions.

(a) With pyrethrum all adults had quick knock-down effect. A small percentage of eggs, larvæ and pupæ developed just up to the next stage and no further.

(b) With BHC treatment also the adults did not survive long. All the pupæ died ; only six per cent of the larvæ developed into adults. Rest of the larvæ died out and none of the eggs developed into adults.

(c) With DDT, adults died within three minutes. All the pupæ died at a little later date than with BHC treatment. Twenty-four per cent of the larvæ pupated but no adults came out of them in this series of experiments. Eighty per cent of the eggs hatched out but the larval period was prolonged considerably in comparison with its usual life-history. None pupated out of them.

NUTRITION

1. Nutrition researches under Dr. V. N. Patwardhan at the Nutrition Research Laboratories, Coonoor.

1. STUDIES ON VITAMINS.

VITAMIN A.

Vitamin A deficiency in the monkey.—The results of the experiment described in the last report in which ocular signs of vitamin A deficiency were produced in monkeys are described below.

Severe damage to the visual cell layer was observed in all the deficient animals. This layer was markedly reduced in thickness and showed alteration in its staining characteristics. Clear-cut histological evidence was obtained to show that the cone cells are as severely damaged as the rod cells in this deficiency in the monkey. There was damage to the outer segments of the cone cells and pyknosis of their nuclei. The pigment epithelium showed striking changes. The pigment which in the control monkeys was limited to the inner (basal) part of the epithelium was consistently found to be irregularly distributed in the epithelium of the deficient animals. Similar changes in the pigment epithelium had previously been observed in avitaminotic rats. No abnormal changes were found in the eyes of the control monkeys which had been fed the same diet as the deficient animals but which, in addition, received a daily supplement of 500 I.U. of vitamin A.

The results of the study indicate that there may be defective cone vision in addition to defective rod vision in vitamin A deficiency. Both in the rat and in the monkey, degeneration of pigment epithelium was found regularly in vitamin A deficiency and appears to be closely associated with the degeneration of the subjacent visual cell layer.

RIBOFLAVIN.

Dietary protein and utilization of riboflavin.—The influence of protein content of the diet on the utilization of riboflavin has been investigated. Rats were fed riboflavin-free diets at 5 and 25 per cent casein level. Controls received as supplement of 500 μ g. riboflavin per 100 g. of the diet. The animals were killed at the end of five weeks. The total riboflavin and FAD in liver were determined. The results are given in Table I :—

TABLE I.

Group	*Total riboflavin μ g./g.	*FAD μ g./g.†
I	15.21 \pm 2.594	14.03 \pm 3.020
II	21.63 \pm 4.469	19.96 \pm 4.803
III	25.83 \pm 5.876	23.39 \pm 7.343
IV	33.60 \pm 4.885	31.73 \pm 5.647

* The results are average values for 14 animals (\pm S.D.)

† FAD expressed in terms of μ g. of riboflavin.

It will be found from the table that there is a significant difference in the levels of total riboflavin and FAD in livers of rats on low protein (five per cent) and high protein diets. Even when 500 $\mu\text{g}/100\text{ g.}$ food were being consumed the riboflavin levels in liver in the former group were lower than in comparable animals on 25 per cent casein diets with and without added riboflavin. These results suggest that on low-protein diets the capacity to utilize riboflavin is adversely affected.

VITAMIN D.

Mode of action of vitamin D.—A few findings on the histochemical changes taking place in the rachitic cartilage of rat after administration of vitamin D have been described last year. Further experiments have confirmed those findings in the main. In addition to these, an alteration in the quality of staining of the matrix in calcifying cartilage has been observed.

In animals treated with vitamin D and showing calcification, a sharp increase in the intensity of staining with periodic acid leucofuchsin was observed in the matrix of the calcifying cartilage in the chondro-osteoid trabeculae undergoing calcification and in the calcified osteoid, as has been previously observed by Rubin and Howard (Trans. 2nd Conf. Metabolic Inter-relations, Josiah Macy, Jr., Foundation, New York, 1950). On staining with pyronin-methyl green, the cartilage matrix in areas showing calcification gave a purplish stain instead of the pink staining found in other areas. In untreated animals rachitic cartilage matrix stained uniformly pink in all regions. In the epiphyseal cartilage of normal growing rats stained with pyronin-methyl green, purple stain is taken by the matrix in the calcifying zone in contrast to pink stain displayed by the matrix in immature and proliferative zones. Thus it appears that the staining characteristics found in the zone of calcification in normal rats are similar to those observed in calcifying rachitic cartilage during healing.

An attempt has been made to interpret the various changes which take place in the rachitic cartilage healing under the influence of vitamin D in terms of a tentative hypothesis to explain the vitamin D action in rickets. It is suggested that in vitamin D deficiency the mechanism of glycolysis or of the oxidative removal of glycolytic products is defective, thus causing an upset in the orderly process of calcification. The above defect probably interferes with the synthesis of the appropriate matrix substance which has a different staining characteristic from that exhibited by the rachitic cartilage matrix. The defective matrix apparently does not permit deposition of the bone salt. Recent observations on the importance of citrate in bone metabolism (Dickens, *Biochem. J.*, **35**, 1011, 1941 and Dixon and Perkins, *Biochem. J.*, **52**, 60, 1952) and the observation that citrate cures rickets (Shohl, *J. Nutr.*, **14**, 69, 1937) can now be brought into line with the suggested hypothesis, since citrate is an important constituent in the tricarboxylic acid cycle of Krebs. The suggested defect in the glycogen metabolism of rachitic cartilage may probably be at the citric acid stage. On administration of vitamin D, the above defect is corrected and the conditions are again made favourable for the synthesis of the ground substance which then permits deposition of bone salt. It is admitted that before one can take this hypothesis as proved, additional convincing evidence with regard to defects in glycogen metabolism and differences in rachitic and normal cartilaginous matrix will have to be brought forward.

Vitamin D content of Indian butter-fat.—Dikshit and Ranganathan (*Ind. J. Med. Res.*, **38**, 37, 1950) had reported very high values (5 to 31 I.U. per g.) of vitamin D for butter-fat obtained from Indian cows and buffaloes. Dr. Kon of the National Institute for Research in Dairying, Reading, U.K., found, on the other hand, that the vitamin D content of the Indian cow butter-fat was not very much different from that of summer butter in the U.K. In view of this, the problem has been re-investigated and it was found that the high values reported by Dikshit and Ranganathan were due to some technical defects in the method of estimation. In fact, Indian butter-fat contains vitamin D at a level comparable to that found in the U.K. summer butter. Full details of the investigation will be published in due course. In the meantime, it was felt necessary to include this note in the report to retract the claims made earlier.

In three samples obtained from Bangalore, values of 1.20, 1.18 and 1.50 I.U. vitamin D per gramme of butter have been obtained.

II. STUDIES ON PROTEINS.

NUTRITIVE VALUE OF DUCK-EGG WHITE.

The results of experiments reported last year proved that the low nutritive value of duck-egg white (DEW) was due to the presence of a powerful tryptic inhibitor contained in it. Further work on this inhibitor is described below.

TRYPSIN INHIBITOR IN DEW.

Attempts were made to isolate the trypsin inhibitor from DEW. It is known that the ovomucoid fraction in egg white contains antitryptic activity, and since it is uncoagulable it can be easily separated from the coagulable proteins of the egg white. DEW was diluted with water (2 : 1) and held at 60°C. for 20 to 25 minutes.

The coagulum was separated and washed with water on a centrifuge. It was used for preparation of diets at nine per cent protein level with other constituents as in earlier experiments. The centrifugate was filtered and to it was added an equal volume of saturated ammonium-sulphate solution. The precipitate formed was centrifuged off and washed with half saturated solution of ammonium sulphate. It was dissolved in water and dialysed for 24 hours, after which the ovomucoid was re-precipitated with acetone, washed with acetone and ether and dried in vacuo (fraction A). The dry powder was added to 0.9 per cent level to the diet containing the coagulable protein. The following results of growth and metabolism experiments in albino rats (Table II) indicate the inhibitory rôle of the ovomucoid on tryptic action and growth :—

TABLE II.
Influence of DEW ovomucoid on growth and digestibility of protein.

Diet containing :	Number of rats	Average gain in weight in four weeks, g.	Digestibility, per cent
(a) Whole DEW ...	6	10.5	66.3
(b) Coagulable portion of DEW	6	40.3	84.4
(c) Ditto— + fraction A ...	6	11.3	67.0

Ovomucoids from DEW and HEW were also prepared by the method of Linweaver and Murray (*J. Biol. Chem.*, **171**, 565, 1947). Proteins of the egg white were precipitated by trichloroacetic acid. To the filtrate were added two volumes of acetone when ovomucoid was precipitated. The precipitate was dissolved in water, dialysed and the ovomucoid reprecipitated with acetone, washed with acetone and ether and dried. These were added at one per cent level to an adequate diet containing ten per cent casein and growth response of rats studied. The results showed that DEW ovomucoid inhibited growth, whereas HEW ovomucoid had no such effect. The effect of DEW ovomucoid addition to diets containing other proteins is being investigated.

Thus the results of experiments described above had shown that the growth-depressing effect and the antitryptic activity were associated with the ovomucoid fraction of DEW. The antitryptic action could be exerted by a general inhibition of proteolysis by trypsin or by specific inhibition of liberation of certain amino acids only. Melnick, Oser and Weiss (*Science*, **103**, 326, 1946) had shown that soya-bean tryptic inhibitor inhibited the liberation of methionine from soya-bean protein as a result of which the availability of methionine was reduced. It was, therefore, considered worthwhile studying the liberation of amino acids by trypsin as affected by DEW ovomucoid.

2,500 mg. casein in a phosphate buffer of pH 8.0 were digested with 100 mg. trypsin at 37° C. To a second flask containing casein and trypsin 25 mg. of DEW ovomucoid were added at the commencement of digestion. Toluene was added as preservative to both flasks. At 24 and 120 hours aliquots were removed, and the amino N determined by formal titration method. In duplicate aliquots withdrawn simultaneously the undigested protein was precipitated by adjusting the pH to 4.2 with acetic acid. In the filtrate, the essential amino acids (except methionine) were determined by the microbiological method. Methionine was estimated colorimetrically. The results of these studies show that the liberation of all the essential amino acids was not hindered to the same extent by the DEW ovomucoid. At 24 hours, valine liberation was affected the most whereas methionine was not affected at all. Marked reduction in the liberation of histidine, threonine and lysine was also observed. Thus it would appear that the availability of essential amino acids is likely to be adversely influenced by the DEW ovomucoid owing to unequal inhibition of rates of their liberation. Work with other proteins is in progress.

PROTEIN METABOLISM IN INDIAN ADULTS.

Efforts to find an explanation for the differences in urinary N excretion observed with vegetable- and animal-protein diets are in progress. As mentioned in an earlier report, small doses of vitamin B₁₂ had failed to show any influence on urinary N of human volunteers kept on vegetarian diets. A fresh experiment with two volunteers to whom a total of 300 µg. vitamin B₁₂ was administered in three days, confirmed the results obtained with smaller doses. A long-term experiment on N balances is under way in an institution where growth trends with and without vitamin B₁₂ are being observed.

Absorption of animal- and vegetable-proteins from the intestines.—The differences in the rates of digestion and absorption of protein were

investigated in adult male subjects on animal- and vegetable-protein diets. It is well known that after a protein meal, the excretion of nitrogen in urine increases during the absorption phase. It was felt that a rough indication of rates of absorption of protein would be obtained if after a standard meal, the hourly urine N excretion was studied up to seven or eight hours. The results of such studies in four adult healthy subjects can be summarized as follows :—

1. In the first few hours after the meal, there was a higher excretion of N in urine on animal-protein diets than on vegetable-protein diets at corresponding periods.
2. The urinary N at peak period was higher on animal-protein diet than on vegetable-protein diet.
3. At the end of eight hours the total urinary N in all the four subjects was higher on animal protein diets than on vegetable protein diets, the difference being 26 to 35 per cent in three cases and 11 per cent in one.
4. About 50 per cent of the total difference in urine N observed in the two diets in eight hours appeared within the first three hours or even less.

These results indicate that probably the vegetable proteins are absorbed more slowly than animal proteins.

Further work is in progress.

THE EFFECT OF AGE ON NITROGEN METABOLISM.

Studies on phenylalanine and cystine in plasma of young and old rats were continued. Phenylalanine was determined by microbiological method and cystine by the method of Brown and Lewis (*J. Biol. Chem.*, **138**, 717, 1941). The results are given in Table III:—

TABLE III.

Cystine and phenylalanine in plasma of young and old rats.

Particulars	Number of rats	Range γ /c.c.	Average γ /c.c.	σ
Phenylalanine at—				
3 months ...	8	2.6–4.5	3.41	0.64
23 „ ...	19	2.8–8.6	4.81	1.71
Cystine at—				
3 months ...	11	10.8–22.3	16.27	2.74
23 „ ...	17	7.7–22.9	13.93	3.89

The results indicate that in old senile rats, the plasma level of phenylalanine is higher and that of cystine lower than the corresponding levels of these amino acids in three-month old rats. The changes in urinary excretion of these amino acids in young and old rats reported earlier thus appear to correspond with those observed in plasma. Similar studies on methionine are in progress.

It must be mentioned that the observed changes in plasma and urine cannot be explained on the basis of the accepted theories of protein metabolism. In view of the fact that very little is known regarding the effect of age on protein metabolism it is difficult to advance a suitable explanation for the observed phenomena. It is possible that with age the tissue-enzyme systems connected with protein metabolism undergo certain changes, the results of which are manifest in the concentrations of amino acids in tissues, body fluids and in urine as well. In order to test whether enzymes are in fact affected by age, the activity of a few liver enzymes was tested in three month old rats and in rats 23 months in age. The findings suggest that some alterations in the activity of the following enzymes can be expected : succinic oxidase, esterase, deaminase and transaminase. On the other hand, butyric oxidase and alkaline phosphatase were found unaffected by age. A more detailed study of this aspect is indicated.

ESSENTIAL AMINO ACIDS IN INDIAN PULSES.

The essential amino-acid composition of five common Indian pulses and one legume has been determined using the microbiological assay technique. The pulses analysed were:—(1) Bengal gram (*Cicer arietinum*), (2) red gram (*Cajanus indicus*), (3) black gram (*Phaseolus mungo*), (4) green gram (*Phaseolus radiatus*), and lentil (*Lens esculenta*). All the amino acids except methionine and cystine were assayed by microbiological methods. Assay of methionine by the above method did not yield satisfactory blanks and the standard curves were not also satisfactory. Methionine and cystine were, therefore, estimated by colorimetric methods. The essential amino-acid contents of the pulses are summarized in Table IV :—

TABLE IV.
Essential amino acids in Indian Pulses.

Amino acid	Bengal gram*	Red gram*	Green gram*	Black gram*	Cow pea*	Lentil*
	Average	Average	Average	Average	Average	Average
Arginine ...	1.51	1.39	1.68	1.44	1.67	1.85
Histidine ...	0.51	0.88	0.72	0.69	0.77	0.57
Isoleucine ...	1.31	1.48	1.58	1.38	1.20	1.46
Leucine ...	1.75	1.81	2.05	1.82	1.84	1.44
Lysine ...	1.39	1.66	1.88	1.52	1.53	1.53
Methionine ...	0.38	0.24	0.28	0.28	0.25	0.20
Cystine ...	0.18	0.21	0.16	0.17	0.17	0.20
Phenylalanine ...	1.09	2.35	1.57	1.37	1.29	1.08
Threonine ...	1.04	0.89	0.95	1.09	0.80	0.77
Tryptophan ...	0.12	0.06	0.12	0.13	0.15	0.09
Valine ...	1.17	1.33	1.72	1.61	1.56	1.42

* Per cent in flour.

It can be seen from Table IV that tyrtptophan, methionine and cystine are the limiting amino acids for the various pulses. The limiting amino acids are, tryptophan for Bengal gram and red gram and methionine

and cystine for green gram, black gram and cow pea. Deficiencies in all the three above amino acids limit the utilization of lentil.

RÔLE OF SULPHUR-CONTAINING AMINO ACIDS IN TOXIC INJURY TO LIVER.

The results of the work carried out thus far on chronic and acute CCl_4 injury seem to indicate that methionine, cystine and glutathione exert protective action, although to varying degrees. In acute injury it was observed that methionine gave the best protection, followed by glutathione and cystine in that order. It is probable that the protective action depends upon the readily available $-\text{SH}$ group in the supplement. This is significant in view of the marked reduction in liver glutathione which was found to take place in the first six hours after an intraperitoneal injection of CCl_4 . It should also be considered significant that among the enzymes investigated those which require a $-\text{SH}$ group for their activity were adversely affected by CCl_4 , whereas others were not so affected.

These observations suggest that the site of action of CCl_4 is the active $-\text{SH}$ group in the liver cell, and supplements such as methionine and glutathione act through their readily available $-\text{SH}$ groups by restoring the activity of certain essential enzyme systems needed for the regeneration of liver.

PLASMA LIPASE AND ESTERASE IN HUMAN MALNUTRITION.

Lipase and esterase activity of blood plasma was determined in adult cases of nutritional œdema soon after admission and after about four to six weeks of treatment. The technique used has been described by Srinivasan and Patwardhan (*J. Lab. Clin. Med.*, **40**, 860, 1952). When the second determination was made on the patients, there had been considerable clinical improvement under treatment with high protein diet. Œdema had completely disappeared. The results are given in Table V (*see overleaf*). Observations on normal adult men are included in the table for comparison.

It will be clear that in adults as in children the plasma lipase and esterase activity is depressed during the active stage of the disease. The response to treatment as judged by restoration of enzyme activity to normal levels, was, however, poor as compared to that obtained in children within a similar period in spite of the fact that clinical improvement had been marked. It is possible that the effect of malnutrition on the enzyme activity of plasma in adults is probably slower in onset, the low values obtained being the result of chronic malnutrition. It is, therefore, to be expected that the restoration of enzyme activity to normal would also be a comparatively slow process. Certain experiments described below reveal differences in the response of young growing albino rats and adult rats when subjected to protein deficiency.

EFFECT OF PROTEIN DEPLETION ON THE LIVERS OF RATS.

Young adult rats over three months old were divided into three groups, each containing 12 males and 12 females. Group I was fed a diet at 20 per cent protein level, casein serving as source of protein; groups II and III were kept on diets with two per cent protein in which the sources of protein were casein and rice, respectively. The other components of the diet were starch, lard, and salt mixture and vitamin supplements

which provided 50 mg. choline per 100 g. diet in addition to other vitamins. Each rat received daily 10 g. of the diet, all of which was consumed. Water was given *ad lib.* Three males and three females from each group were killed at the end of 30, 60 and 90 days. The livers were removed and freed of blood. A small portion was taken for histological examination and the rest suitably divided for the following estimations : moisture, total N, glutathione and the following enzymes : (a) succinic oxidase, (b) butyric oxidase, (c) alkaline and (d) acid phosphatases, (e) transaminase, (f) d-amino acid oxidase, and (g) esterase. Endogenous respiration of liver was also studied in Warburg micro-respirometer.

At the end of 90 days, surviving rats of the two protein-deficient groups were divided into two sub-groups each. Two of the sub-groups were continued on the respective casein and rice low-protein diets and two others were fed 20 per cent protein diet similar to that received by the control animals. All the rats were sacrificed at the end of 120 days from the start of the experiment. Livers were treated as mentioned above.

The findings of biochemical investigations at 30 days and 120 days are given in Table VI :—

TABLE VI.
Effect of protein depletion on liver enzymes and other components.

Particulars	Control—20% protein (casein)		2% protein (casein)		2% protein (rice)	
	After days		After days		After days	
	30	120	30	120	30	120
Body-weight, g.	159	205	139	175 (183)	148	142 (225)
H ₂ O/100 g. liver, g.	64·8	69·31	65·6	70·75 (69·34)	65·13	69·47 (69·46)
Total N/100 g. liver, g.	3·750	3·635	2·631	2·368 (3·38)	2·561	2·411 (3·39)
Glutathione/100 g. liver, mg.	144	194	43	42 (204)	54	49 (204)
CFA/100 g. liver, g.	3·12	...	3·67 (3·29)	...	3·91 (3·59)
Succinic oxidase/g. liver	43	46	16	18 (42)	16	18 (46)
Butyric oxidase/g. liver	12·3	12·1	2·2	3·1 (10·9)	2·0	4·0 (13·7)
Alkaline phosphatase/100 g. liver	54	51	142	174 (58)	109	132 (56)
Acid phosphatase/100 g. liver	458	445	456	448 (437)	450	476 (429)
Transaminase/g. of liver	147	153	95	115 (148)	86	109 (143)
D-amino acid oxidase/g. of liver	507	529	422	25·3 (480)	458	203 (473)
Esterase/100 mg. liver	258	323	223	173 (323)	223	137 (333)
QO ₂ μ l/hr./mg. „	5·13	5·55	2·62	3·10 (5·11)	2·68	3·64 (5·33)
Lipase/100 mg. pancreas	250	...	83 (271)	...	40 (263)

Notes :—Figures in parentheses are for re-alimented rats. For definition of enzyme units original papers may be consulted.

In protein-deficiency states the following effects were seen : (1) The total N and reduced glutathione of liver showed a significant fall in the first month, with little change thereafter ; (2) crude fatty acids seemed to increase slightly up to two months, but showed a fall thereafter bringing the values nearer those found in the control animals ; (3) succinic and butyric oxidases, transaminase, d-amino acid oxidase and esterase showed a marked fall in activity due to protein deficiency. In the last two enzymes the fall was only significant at 60 days, whereas in others almost the minimum values were obtained within 30 days ; (4) alkaline phosphatase showed a large increase in activity ; (5) acid phosphatase seemed to be unaffected except for values obtained in deficient animals at 90 days which were distinctly higher than controls.

During thirty days of re-alimentation, total nitrogen and glutathione in liver and the activity of various enzymes had all returned to levels comparable to those existing in the control animals. Thus it would appear that in spite of protein depletion for 90 days, the effects could be rapidly reversed.

A similar experiment with young rats (four weeks old) was conducted but for a shorter duration. The results indicate that in young growing animals the effects of protein deficiency are much quicker in onset and more severe than in adult animals of the species.

The histopathological examination of livers obtained in these experiments is still incomplete and will be reported on later.

III. THE ALLEGED TOXICITY OF PULSES.

Pulses form an important constituent of the Indian dietaries. Results of Diet Surveys (*I.C.M.R. Special Report No. 20*) have shown the average intake to be in the neighbourhood of two ounces per consumption unit per day. The Nutrition Advisory Committee (1944) has recommended the inclusion of 3 oz. pulses per day per consumption unit. Apart from serving as rich sources of protein, they also supply appreciable amounts of the B-vitamins. It was, therefore, somewhat disturbing to find a report by Pal and Bose (*Ind. Med. Gaz.*, **78**, 436, 1953) in which it was stated that almost all the pulses in common use in India, when included at 33 per cent level in a diet based on rice, proved toxic causing severe injury to livers and kidneys of the experimental rats. The authors unfortunately gave no information of the type of damage suffered by these organs. These observations fortunately have not received much notice and rightly so, for pulses have been used as human food in India and no ill effects such as damage to liver and kidney have come to notice during their long use by millions of people in this country. In view of the fact that liver damage in albino rats had been ascribed to the pulse in the diet, it was considered desirable to verify the claims of Pal and Bose. All the common pulses have been tested in rats at 33 per cent level in a diet composed of pulse, rice and NaCl, identical to that used by Pal and Bose. In no case could any damage to liver or kidney be demonstrated. The above diet is markedly inadequate in calcium and phosphorus and as a result retardation of growth, paresis of hind limbs and low bone ash were observed in rats kept on pulse-rice diets. All these effects could be prevented by raising the levels of calcium and phosphorus in the diet. Feeding of pulses even at the very high level of 66 per cent has not resulted in injury either to liver or kidney.

Thus, pulses can be considered not to be toxic ; the reason for toxic effects described by Pal and Bose must probably lie outside the dietary constituents used.

IV. NUTRITION CLINIC.

During the year, 3,725 cases attended the Clinic. Of these 153 cases were found to be suffering from nutritional disorders (this includes 32 cases from Coimbatore). The distribution of nutritional diseases is given in Table VII. From among these, the cases of nutritional oedema in adults and nutritional-oedema syndrome in children continued to receive special attention for purposes of intensive investigation.

TABLE VII.

Statement of in-patient and out-patient cases investigated and treated during the year ending 31st March, 1953.

Type of deficiency	Presenting feature	In-patient	Out-patient
Vitamin A ...	Night-blindness only	11
	Xerosis and/or Bitot's spots	48
	Keratomalacia ...	3	4
Mixed deficiency	B-complex deficiency		
	(a) Burning-feet syndrome	3
	(b) Angular stomatitis, cheilosis and glossitis	10
	(c) Pellagra ...	1	1
	Nutritional oedema ...	29	...
	Nutritional oedema Syndrome ...	30	6
	Marasmus ...	1	...
	Rickets ...	1	1
Anæmia ...	Iron-deficiency anæmia ...	2	1
	Megaloblastic anæmia ...	1	...
Total	68	85

Total attendance for the year ... 3,725.

INVESTIGATIONS ON NUTRITIONAL OEDEMA.

During the last two years considerable progress has been made in the study of nutritional oedema. Results of observations and experimentation on such aspects as (a) body composition, (b) electrolyte metabolism and (c) pathogenesis have been described in detail in the earlier reports. During the last year work on some of these aspects has been continued. Observations on the effect of malnutrition on energy metabolism are described below.

Basal metabolism in nutritional oedema.—An important physiological adaptation to the restricted calorie intake during semi-starvation is reported to be a fall in basal metabolism. This could result from (1) a reduction

in metabolising tissue due to wasting, and (2) a reduction in oxygen consumption per unit of metabolising tissue as suggested by Keys. The changes in B.M.R. accompanying nutritional œdema and those following on rehabilitation were studied in order to find out whether either or both of the above mentioned factors were operative in determining the level of B.M.R.

The B.M.R. was determined in nine cases of nutritional œdema immediately on admission, and again after the disappearance of œdema. The time interval between the determinations was about 25 days. Using a Benedict-Roth Metabolism Apparatus, two runs of eight minutes each were recorded at an interval of ten minutes and the average of the two observations which did not differ by more than five per cent was used for calculating the B.M.R. On the same day, the body composition of the patient was also determined according to the method already described. From the cellular solids thus estimated, the oxygen consumption per kilogram of cell solids was calculated. The results are given in Table VIII:—

TABLE VIII.

Basal metabolism in cases of nutritional œdema, at the time of admission and after rehabilitation.

No.	B.M.R. in calories/hour				B.M.R. in O ₂ consumption per kg. of cell solids/min.	
	Absolute value		Per square meter			
	Before	After	Before	After	Before	After
1	37·3	45·7	28·9	32·2	21·8	22·5
2	49·6	56·7	29·3	35·9	21·8	22·6
3	44·4	49·1	30·6	33·1	22·3	22·7
4	32·7	42·1	23·5	31·1	22·4	22·5
5	44·5	47·4	27·9	32·6	21·6	22·5
6	47·2	57·3	30·9	38·9	21·6	22·3
7	36·3	43·1	23·7	32·2	20·8	23·3
8	44·8	47·5	34·4	34·6	22·4	21·9
9	36·3	41·2	27·1	33·0	21·2	21·8

Basal metabolism expressed in terms of surface area : Expressed as calories per hour per square meter, the B.M.R. ranged from 23·5 to 34·4 calories at the time of admission and averaged 28·5 calories. After rehabilitation the B.M.R. showed a rise, ranging from 31·1 to 38·9 calories, being 33·7 calories on the average.

The B.M.R. estimated on admission was, therefore, found to be below normal by about 21 per cent as compared with the reported average of 35 calories per square meter per hour for normal Indian adults. After treatment, the B.M.R. was higher than at the time of admission, but had not come up to the normal level. There was an absolute rise in basal metabolism. Since in every case at the end of treatment there was also a concomitant reduction in the body surface area, the B.M.R. showed the appreciable rise reported above.

(b) Basal metabolism expressed in terms of cell solids : The oxygen consumption per kilogram of cell solids ranged from 20·8 to 22·4 c.c. per minute at the time of admission, and from 21·8 to 23·3 c.c. per minute after the disappearance of œdema.

It will be seen from the above that in spite of the marked rise in the B.M.R. after treatment when correlated to the surface area, the oxygen consumption per kilogram of cell solids was found to be almost unchanged—21·8 c.c. per minute (average) on admission and 22·5 c.c. per minute (average) on rehabilitation.

It is concluded from these observations that :

- (i) At the height of œdema, cases of semi-starvation have subnormal B.M.R.
- (ii) There is a rise in the B.M.R. after rehabilitation, and
- (iii) The reduction in the B.M.R. at the height of œdema is almost entirely due to a reduction in the active 'cell mass' and not to any reduction in the oxygen consumption per unit of metabolising tissue.

Electrocardiograms in nutritional œdema.—Whereas a great deal of attention has been devoted to the study of the state of liver and pancreas in protein and caloric malnutrition, other organs appear to have been comparatively neglected. The experience of Davies in Uganda suggests that heart is also affected to a certain extent.

In the present study electrocardiographic changes in 20 cases of nutritional œdema in adults and ten of nutritional-œdema syndrome in children were observed on admission and after treatment. The abnormalities in the electrocardiograms at admission were of four main types : (1) bradycardia of sinus type in a majority of cases, (2) a marked diminution in the amplitude of all the deflections, the T-waves were quite often either isoelectric or inverted in both the limb leads as well as the V-leads, (3) a relative prolongation of the QT interval, and (4) irregularities of rhythm in a few cases.

The above changes were not influenced by vitamin E or B₁ therapy. On the other hand, treatment with high-protein diets reversed the abnormalities except the relative prolongation of the QT interval. It is felt that myocardial damage may be responsible for the electrocardiographic changes in nutritional œdema. In two cases seen at autopsy myocardial involvement like brown atrophy and myocardial fibrosis were observed.

NUTRITIONAL-ŒDEMA SYNDROME IN CHILDREN.

The study of the dietary background which contributes to the evolution of the syndrome was continued. The dietary history of patients brought to the clinic was obtained from the parents by careful questioning. The data thus collected on 25 cases were analysed for the nutrient and calorie contents with the help of *Health Bulletin No. 23*. The results are presented in Table IX. A parallel field investigation in which diet surveys by weighment method were done in the homes of patients has been described elsewhere in this report. It will be clear that the questionnaire method has given a fairly correct picture of dietary deficiencies in children suffering from nutritional-œdema syndrome.

TABLE IX.

Mean intake of various nutrients in 25 cases of nutritional-œdema syndrome in children.

No.	Age, years		Calories		Carbohyd- rate, g.		Fat, g.		Proteins, g.		Calcium, mg.		Phosphorus, mg.		Iron, mg.		Thiamine, µg.	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
25	21½	10/12	758	399	161	89	3	1·0	14	7·4	69	18	173	31	6	2·4	429	130
		to		to		to		to		to		to		to		to		to
		4 yrs.		1252		286		6·5		28		176		520		10		776

VI. PATHOLOGY.

Histochemical techniques.—The advantages of using polyethylene glycols as embedding media in histochemical work have been described previously. In addition to the components which have been successfully demonstrated in sections, DOPA melanase has been found to be well preserved in sections of skin prepared by this method.

A quantitative study of the preservation of alkaline phosphatase in tissue sections prepared by the polyethylene-glycol method has been undertaken.

V. FIELD WORK.

Field studies on nutritional-œdema syndrome.—Field investigations were commenced with the object of collecting information on the socio-economic and nutritional background of the class of families from which a majority of cases of nutritional-œdema syndrome are derived in order to study the predisposing factors and the evolution of the disease. This study has been undertaken among the labour population on the neighbouring tea estates.

An analysis of 143 families (only families with one or more children were included) revealed an average family size of 4·7 members (range 3 to 11). The average daily income was found to be Rs. 0·7-4 per head per day.

Majority of families were living in quarters provided by the estate management, the accommodation being just sufficient. The drinking water was pumped from the local springs and delivered by pipes at the dwelling places. Environmental sanitation was very unsatisfactory, since these people did not use the latrines that were provided. Both men and women worked seven hours a day. Most of the women, when pregnant, continued to work in the tea-gardens almost till the day of delivery. For eight weeks after delivery, the mothers stayed away from work and received a maternity benefit of Rs. 4-8-0 per week.

A diet survey was carried out on 20 families comprising 113 individuals for a period of seven days by weighing-of-food method. The average intake per consumption unit is given below :—

	Oz.
Cereals—	
Rice	7·4
Cholam	11·0
Pulses	1·2
Fats and oils (mainly ground-nut oil)	0·3
Jaggery	0·8
Vegetables—	
Tuberous	0·5
Non-leafy	0·9
Leafy	—
Beef	0·7

The calorie content of the diet was 2,225 and protein level was at 62 g. The diet was deficient in fats, calcium and vitamin A.

Information on the feeding and care of infants has thrown valuable light on the ætiology of nutritional-œdema syndrome.

Most of the infants were reported to be normal and healthy at birth. In the first eight weeks when mothers are not at work the infants are fed on demand at breast and are usually well looked after. The health of the child is apparently satisfactory during this period. When the mothers go back to work after eight weeks, the infant is either left at the creche where one is provided or more often it is left to the care of other older children. The infant receives three feeds at breast during the day and on demand during the night. Till about six months, the infant receives diluted cow milk as supplementary feeds during the day. After this, cow milk is discontinued and the child is put on gruel or has to partake of the food prepared for adults. Children continue with breast feeding for about two to three years, but the nutrition which they receive in this way must be very meagre.

When mothers go off to work, they usually leave some cooked rice and soup in the house. This is eaten by the child off and on within the next one or two hours during which period it is open to contamination by flies. This very frequently exposes the child to various gastro-intestinal infections and infestations. Accurate information of food consumption was obtained by hospitalizing 14 children of one of six years and collecting all food which the mothers gave to these children during the 24-hour period. Twelve of these children exhibited clinical oedema and were considered as cases of nutritional-oedema syndrome (Kwashiorkor). Replicate samples of food were taken and analysed in the laboratory. In the case of children still at breast, 24-hour collections of milk were obtained and the nutrient content taken into consideration when calculating the daily intake.

The results showed that the diets were grossly deficient in calories and protein. The calorie intake varied from 233 to 697 calories, whereas for the same age groups the optimal recommended intake is 900 to 1,400 calories. Similarly protein intake was also 50 per cent or less than the recommended figure for the corresponding age groups. Other deficiencies also existed but they were overshadowed by the calorie and protein deficiency seen in these malnourished children. It is interesting that the observations regarding the dietary intake of these children arrived at by elaborate analysis of the foods actually consumed, correspond closely to the conclusions arrived at by the oral questionnaire method in the clinic.

A detailed history of the development of the disease was obtained in 12 cases that were encountered during this study. As stated earlier, the infants were reported to be healthy and apparently normal at birth. The disease made its appearance usually in the latter half of the first year or subsequently. In all respects, the development of the disease followed the pattern described by Trowell and others. The dietetic and other details elicited in the present study support the concept that the disease is primarily caused by calorie and protein malnutrition in which gastro-intestinal infection and infestation probably play a contributory rôle.

Studies on the breast milk of Indian women.—In their report on 'Kwashiorkor' in Africa. Brock and Autret (*WHO Monograph No. 8, 1952*) refer to some work on the composition of breast milk of African mothers. The figures quoted there reveal that fat : protein ratio in the milk of African mothers was very high with the result that there was a nutritional imbalance in the milk constituents. The protein content of milk, however, appeared to be approximately the same as reported in European women.

There are a few analyses of breast milk in India. It was felt that further information on this aspect would be desirable in understanding the ætiology of Kwashiorkor.

So far 29 specimens from labour-class women in the Nilgiris have been analysed. Of these 20 specimens were from apparently healthy women and nine from mothers whose children suffered from nutritional-œdema syndrome. The results are given in Table X:—

TABLE X.

Composition of breast milk.

	G. per 100 c.c. of milk			
	Protein	Lactose	Fat	Total solids
A. Apparently healthy women (Nilgiris)—20				
Range ...	0·90–1·91	6·00–7·46	1·84–8·30	11·00–14·37
Average ...	1·19	6·91	4·11	12·03
B. Apparently healthy women (Nilgiris) mothers of children suffering from nutritional-œdema syndrome—9				
Range ...	0·99–1·46	6·72–8·34	2·04–8·31	11·04–14·53
Average ...	1·24	7·30	4·44	12·76
C. Healthy European mothers—(Mavis and Gunther, <i>Brit. Jour. Nutr.</i> , 6, 2, 1952)				
Average ...	1·20	6·5	3·6	12·03

It will be clear from the above table that the composition of Indian breast milk does not differ appreciably from that reported for the breast milk of European women. Since the number of specimens was small and obtained at varying periods of lactation, it is not possible to make more exact comparison with the reported data on African mothers' milk. The results indicate, however, that the composition of breast milk of mothers of children suffering from nutritional-œdema syndrome is almost identical with that obtained for breast milk of mothers of healthy children. Much larger number of milk samples need to be analyzed in a more comprehensive manner before the nutritive value of Indian breast milk can be fully evaluated. One other aspect, however, has received attention. Aufret and Tanguy (1949, quoted by Brock and Autret, 1952) found a lower value (average 0·164 g./litre) for methionine in maternal milk of Africans at Dakar than has been reported (average 0·290 g./litre) for the milk of European mothers. In the absence of fuller details, it is difficult to evaluate the significance of this report. The estimation of methionine in milk

presents some difficulties and as Brock and Autret mention the above authors did not test the milk of European mothers as a control for their method.

A careful estimation of methionine and cystine in 25 specimens of breast milk of Indian women in Coonoor has given the following results (Table XI) :—

TABLE XI.

Methionine and cystine in human-milk protein.

	Methionine g. per 16 g. N.		Cystine g. per 16 g. N.	
	Range	Average	Range	Average
Breast milk, Coonoor	2·03–3·99	2·91	1·31–3·31	1·88
American women (Block and Bolling— <i>Arch. Biochem.</i> , 10 , 359, 1946)	...	2·28	...	2·78

Further work is in progress.

2. Nutrition research unit under Dr. B. C. Guha at the University College of Science & Technology, Calcutta.

SYNOPSIS.

Studies on the biosynthesis of ascorbic acid.—Both from *in vitro* experiments and *in vivo* studies by the tracer technique it appears that pyruvic acid is a precursor of ascorbic acid for its synthesis by the rat. The C¹⁴ of labelled pyruvamide appeared in the ascorbic acid isolated from the urine of rats. Phosphorylated intermediary compounds, acids of the Szent-Györgyi and Krebs cycle and various amino acids present in the liver tissue of rats fed with labelled pyruvamide have been separated by the paper chromatographic technique and the radio-autographs of these compounds indicate that C¹⁴ has not been incorporated in any of them.

Biochemical studies on nicotinic acid.—It was reported last year that the bound nicotinic acid was concentrated from rice bran to the extent of about 50 times. This work was followed up during the year under review. As a result of this about 70-fold concentration has been obtained. The starting material contained about 0·3 mg. of bound nicotinic acid per gramme. The final precipitate which was brownish in appearance contained about 20 mg. of bound nicotinic acid per gramme.

Studies on the metabolism of iron during the embryonic development of hen's egg.—The volume per cent oxygen capacity of blood of the chick at different stages of embryonic development was determined. It was observed that volume per cent oxygen of the chicken blood increased from tenth day and reached a maximum on the 21st day of incubation.

Effect of canning and storage on the nutritive values of some common Indian vegetables.—Cabbage lost about 30 per cent ascorbic acid during balancing and holding the blanched samples exposed to air for one hour. The

total loss of ascorbic acid in cabbage up to the end of cooking (autoclaving) operation came to about 92 per cent. Of the remaining vitamin in the can about 39 per cent was found in cabbage and 61 per cent in brine. The effect of storage on the destruction of ascorbic acid was not appreciable. During autoclaving about 36 per cent ascorbic acid was destroyed in the total can content to which synthetic ascorbic acid had previously been added. After three months' storage about 45 per cent ascorbic acid was retained, of which about 25 per cent was in cabbage and 75 per cent in brine. Studies on cauliflower and parwar (*coccinia indica*) are in progress.

Thiamin values of pure-bred strains of cereals and pulses.—Thiamine content of about 175 samples of pure-bred strains of cereals and pulses obtained from different States have been estimated. The same samples obtained from the succeeding years crop are being analysed for their thiamin content. Thiamin values of the samples analysed so far are in good agreement in two successive years crops.

STUDIES ON THE BIOSYNTHESIS OF ASCORBIC ACID.

Precursor of ascorbic acid : Pyruvic-acid and ascorbic-acid formation in vitro.—From the study of the factors influencing the biosynthesis, it was suggested that pyruvic acid or some product of its metabolism is the probable precursor of ascorbic acid. To investigate this point, some *in vitro* experiments with pyruvate as substrate and liver tissue as the source of enzyme were undertaken.

Experimental.—Male albino rats, weighing 150 g. to 200 g., maintained on a basal stock ration, were used throughout the experiments.

Treatment of the tissue.—The animals were sacrificed by stunning. The liver tissue was taken out, pressed between folds of filter-paper and immediately transferred to a mortar kept previously cooled by ice-salt mixture. The tissue was allowed to cool for nearly 15 minutes, then mildly macerated and homogenized. Livers from different animals were taken together and minced. Lots of 2 g. of weighed tissue were taken in glass-stoppered flasks of 30-c.c. capacity. The ratio of the tissues to the incubating fluid employed was 1 : 3. The incubation was carried out in a Ringer-phosphate buffer of pH 7.4, with or without substrates for three hours in an incubator adjusted to temperature of $38^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$. During the incubation, flasks were well stoppered having air inside the flask and no shaking was allowed during the entire period of incubation to prevent the further oxidation of ascorbic acid formed.

Sodium pyruvate was prepared according to the method of Robertson (*Science*, 1942, **96**, p. 93). Results are given in Table I which also shows the substrates used :—

TABLE I.
Synthesis of ascorbic acid from pyruvate in vitro.

Substrate	Per cent increase of ascorbic acid
Pyruvate	17.3
Pyruvate and vitamin B ₁	30.5
Pyruvate and chloretone	25.5
Pyruvate, chloretone and vitamin B ₁	32.2

It will appear from Table I that there is a definite increase in ascorbic-acid content under the stated conditions of experiments, maximum being in the case of pyruvate with added chloretone and B₁.

*Studies by the tracer technique, using labelled pyruvamide ($\text{CH}_3\text{C}^{14}\text{OCONH}_2$).—*The *in vitro* experiments indicated that pyruvic acid might be a probable precursor of ascorbic acid. In order to get more conclusive evidence, the tracer technique was applied for the study of this synthesis *in vivo*. Isotopic pyruvamide in suitable daily doses was administered to rats under chloretone and the excreted ascorbic acid, isolated as such or its derivative, from the urine of rat was tested for radio-activity. It was observed that C¹⁴ from the labelled pyruvamide had been incorporated in the ascorbic acid molecule synthesized by rats under chloretone.

Experimental.—Four male albino rats (150 g. to 200 g.) were maintained on a basal diet of whole wheat, milk, yeast powder and cod-liver oil in adequate proportions and water *ad libitum*. All the rats were housed individually in metabolism cages from the beginning of the experiment. The rats were given chloretone daily by mouth at 20-mg. level per 100 g. body-weight, the vehicle being olive oil. Twentyfour hours urine was collected in 5 c.c. of ten per cent oxalic acid and when the excretion figure reached a maximum (generally on the fourth day) the animals were supposed to be ready for experiment.

At this stage every rat was injected intraperitoneally with roughly 2.5 mg. of C¹⁴-pyruvamide in 1 c.c. of water and having an activity of 1.7×10^7 counts per minute. Such pyruvamide injection was repeated for two more days successively.

Collection of urine.—Urine was collected as stated before and kept in cold storage and combined at the end of the injection period. The collected urine was diluted to a definite volume with water, and the total urinary ascorbic acid was found to be 210 mg. by the indophenol titration.

Ascorbic acid and its di-nitro phenylhydrazine derivative were isolated from the urine of treated rats and obtained in pure crystalline form as described in the previous report. They were then subjected to test for the measurement of their radio-activity.

Measurement of radio-activity.—All materials, assayed for radio-activity, were mounted on pieces of filter-paper fixed on brass holders. Counting was done by the Geiger-Müller counter. The counter used was a screen-wall counter (Libby type). The diameter of the cathode was 1", the central wire 4 mm. The counter was filled with argon and alcohol vapour mixture. In the determination of activity no corrections for self absorption or back scattering were made, but as all the samples were mounted on the same type of mounts and counted with the same geometrical efficiency, these errors will be common to all and would consequently be largely eliminated.

Testing for C¹⁴ in the isolated ascorbic acid and di-nitrophenyl hydrazine derivative from the collected urine.—The specific activities of ascorbic acid and its di-nitro phenylhydrazine derivative from the urine of the rat was found to be 315 c.p.m. and 110 c.p.m. per mg., respectively. The radio-active purity was established by the constancy of the specific activity through several successive re-crystallizations.

Testing for C¹⁴ in the isolated phosphorylated intermediary compounds from the liver tissue of rat.—At the end of the experimental period three rats were

killed and their liver excised. The phosphorylated intermediary compounds were isolated chemically according to Umbreit *et al.* 'Manometric Technique' 1947, p. 183) as described in the previous report. Then the two fractions obtained by fractionation of the trichloroacetic acid extract of the liver tissue were subjected to tests for radioactivity. No detectable counts were given by any of the fractions containing the mixture of phosphorylated intermediary compounds.

Testing for C^{14} in isolated glycogen from rat-liver tissue.—Glycogen was isolated from the liver tissue of the fourth rat according to the method of Good *et al.* (*Jour. Biol. Chem.*, 1933, **100**, p. 485). The radioactivity of the isolated glycogen was measured and found to give 70 c.p.m. per mg. Glycogen was re-precipitated several times before the measurement of its radio-activity.

The results obtained from radio-active measurements are summarized in Table II :—

TABLE II.

Measurement of radio-activity of different substances isolated from the urine and liver tissue of treated rats.

Substance isolated	SPECIFIC ACTIVITY : Counts per minute per mg.
1. Ascorbic acid	315
2. 2 : 4-di-nitrophenylhydrazine derivative of dehydro-ascorbic acid	110
3. Phosphate esters :	
(i) ' Barium-insoluble fraction ' ...	<i>Nil</i>
(ii) ' Barium-soluble alcohol-precipitable fraction	<i>Nil</i>
4. Glycogen ...	70

Following up of the C^{14} of pyruvamide in other metabolites :

The C^{14} of the pyruvamide was followed up through

- (i) The formation of phosphorylated compounds.
- (ii) Acids of the Szent-Györgyi-Krebs cycle.
- (iii) Tissue amino acids.

with the help of paper chromatography and radio-autography according to the method of Fink *et al.* (*Nature*, 1947, **160**, p. 801) and Benson *et al.* (*Jour. Amer. Chem. Soc.*, 1950, **72**, p. 1710).

It was observed that none of the individual phosphorylated intermediary compounds, acids of the Szent-Györgyi-Krebs cycle and amino acids isolated from the liver tissue of treated rats had incorporated any C^{14} in their respective molecules.

Preparation of the tissue extract.—At the end of the experimental period, i.e. after the collection of urine for the isolation of ascorbic acid, three rats were killed by giving blow on the head. They were then decapitated and the liver excised. Pooled livers were homogenized with acetone and

treated for the isolation of the acids of the Szent-Györgyi-Krebs cycle according to the method of Frohman *et al.* (*Jour. Biol. Chem.*, 1951, **193**, p. 277).

The homogenate left after extraction of the acids was treated with sufficient amount of ten per cent trichloro-acetic acid. The phosphorylated intermediary compounds were isolated from the trichloro-acetic acid extract and amino acids were finally isolated by autoclaving the protein precipitate left after the trichloro-acetic acid extraction.

PAPER CHROMATOGRAPHY.

(a) Separation and identification of phosphorylated intermediary compounds from rat-liver tissue by the circular paper chromatography.

Experimental : Apparatus.—The apparatus used by Giri *et al.* (*Jour. Ind. Inst. Sci.*, 1952, **34**, p. 95) was assembled from two Petri-dishes of the same size, one being inverted over the other. Within this combination was kept a small sized Petri-dish which contained the solvent for development.

Paper.—Whatman No. 1 filter-paper of 18.5 cm. diameter was used throughout the investigation. Papers were used as such without any treatment.

Spray reagent.—Hanes and Isherwood's (*Nature*, 1949, **164**, p. 1107) reagent as modified by Burrows *et al.* (*ibid*, 1952, **170**, p. 800) was used. One gramme of ammonium molybdate was dissolved in 8 c.c. of water and 3 c.c. of concentrated HCl; 3 c.c. perchloric acid (70 percent) were added and the mixture was diluted to 100 c.c. with acetone.

Solvent.—Out of mixtures of solvents tried, the following solvent mixture was found best for the resolution of the esters by the circular paper technique: n-butanol, 40 vol.; acetic acid (glacial), 10 vol.; and water, 50 vol. The top layer was used for the development of the chromatogram. This solvent mixture must be freshly prepared since the composition of the phases changes upon gradual esterification of the ingredients of the mixture.

Developing procedure.—5 μ l. to 10 μ l. of the solution containing 5 μ g. to 10 μ g. of each of the phosphate esters in the form of their sodium salts were delivered from a capillary tube to the centre of the filter-paper, the size of the spot being limited to 0.5 cm. in diameter. When multiple application of a dilute solution was found necessary, the spot was air-dried each time after application. The method of development of the chromatogram was the same as described by Giri *et al.* (*loc. cit.*).

The development was carried out at the room temperature. The solvent front travelled a distance of about 8 cm. to 9 cm. in about 1½ to 2 hours. After development the paper was air-dried at room temperature (27° C.). Multiple development of the chromatogram as described by Giri *et al.* (*loc. cit.*) was found to give better resolution.

Detection and identification of phosphate esters.—Inorganic phosphate appeared immediately after spraying the molybdate reagent (Burrows, *et al.*, *Nature*, 1952, **170**, p. 800) on the air-dried paper. In order to

detect the phosphate esters, immediately after spraying the reagent, the paper was kept in an electric air oven at 50° to 60° C. for a period of 20 to 25 minutes for the completion of the hydrolysis of the esters. The blue concentric rings gradually appeared. Then the paper was kept overnight at room temperature when the whole paper turned blue and was next exposed to ammonia vapour for 30 seconds. The blue background disappeared and distinct well-defined blue rings due to different phosphate esters appeared.

The phosphate esters were identified from the measurement of the radii of the arcs produced by them or in other words from their Rf values.

TABLE III.

Rf values of phosphate esters.

Compounds	SOLVENT MIXTURE :	
	n-butanol : acetic acid : water	
Glucose-1-phosphate	0·18
Glucose-6-phosphate	0·31
Fructose-1-6-di-phosphate	0·34
dl-glyceraldehyde-phosphate	0·60
Di-hydroxy-acetone-phosphate	0·67
3-phosphoglyceric acid	0·20
Phosphopyruvic acid	0·27
Adenosine-triphosphate	0·23
Adenosine-5-phosphate	0·32
Di-phosphopyridine nucleotide	0·12
Orthophosphate	0·38
Pyrophosphate	0·21

None of these substances showed any radio-activity.

The identification of amino acids present in rat-liver tissue by the circular paper chromatographic technique.—The precipitated protein mixture obtained after extracting liver with ten per cent trichloro-acetic acid was autoclaved for a period of six hours at 15-lb./sq. in. pressure with a solution of 6N HCl twenty c.c. of 6N HCl were used for each 2 g. of precipitated proteins. After autoclaving the solution was filtered. This solution was then chromatographed. The procedure employed in this case was that adopted in the chromatography of phosphate esters. After the completion of development, the paper was air-dried, treated with H₂O₂ and molybdate to ensure oxidation (Dent, C. F., *Biochem. Jour.*, 1948, **43**, p. 169) and sprayed with a solution of 0·1 per cent ninhydrin in absolute acetone. The paper was then heated at 60° C. for a period of ten minutes when all the rings due to amino acids appeared. The amino acids were identified by comparing them with the known standard chromatogram.

The identification of carboxylic acids of the Szent-Györgyi and Krebs cycle in rat-liver tissue by the circular paper chromatography.—The method followed for the isolation of the acids from the liver tissue of rat was that of Frohman *et al.*

(*Jour. Biol. Chem.*, 1951, **193**, p. 277). The liver tissue was taken out immediately after killing the rat and frozen in solid CO₂. The frozen tissue was homogenized in a Waring-Blender with cold acetone. After adjusting the pH of the resulting homogenate to 3.0 with HCl, it was allowed to stand overnight at 4° C. The solution was then filtered and an aliquot was evaporated to 5 c.c. and then filtered to remove fat. Quantitative transfers were ensured by repeated rinsings with small amounts of water. Acetone was added to the filtrate to facilitate evaporation almost to dryness, and this was accomplished without heating by directing a stream of air from an electric fan over the solution.

Developing procedure.—This solution was then spotted at the centre of the circular filter-paper and chromatographed. The paper was then air-dried and sprayed with the bromophenol indicator prepared according to the method of Lugg and Overall (*Nature*, 1947, **160**, p. 87). The rings obtained due to different acids were identified by comparing them with the respective standards.

Radio-autography of the chromatographed substances.—Radio-autography of the paper chromatograms was done according to the method of Fink *et al.* (*Nature*, 1947, **160**, p. 801) and Benson *et al.* (*Jour. Amer. Chem. Soc.*, 1950, **72**, p. 1710). After the development of the individual chromatogram it was dried in air. Then the filter-paper disc (18.5 cm.) was placed on individual x-ray films and kept in direct contact by pressing them in photographic frames and left in a dark room. Sufficient time, one month, was given for the β -particles emitted from C¹⁴, if any, to affect the x-ray films. During this period test samples were withdrawn, developed and examined for the radiation effect. Finally, at the expiry of the period all the x-ray films were developed for the detection of the black rings on the film.

It was observed that no black ring appeared on the x-ray films with any of these chromatograms. Neither the phosphorylated intermediary compounds, acids of Szent-Györgyi-Krebs cycle, nor the amino acids present in the liver tissue were found incorporated with C¹⁴ in their respective molecules.

BIOCHEMICAL STUDIES ON NICOTINIC ACID.

The purification of this compound was attempted with the help of the following techniques :—

- (a) Fractional precipitation with different solvents.
- (b) Column chromatography.

The final procedure adopted is briefly described below :—

Fifty gm. of raw rice-bran (fresh) were extracted with dilute hydrochloric acid on a boiling water-bath. The extract thus obtained was precipitated with three times the volume of acetone, left at the room temperature for ten minutes and then centrifuged. The precipitate which contained very little bound nicotinic acid was discarded. The supernatant liquid containing the major portion of the substance was again precipitated with acetone up to the concentration of 12 times with respect to the extract originally taken. The whole mixture was left at room temperature for 30 minutes and then centrifuged. The precipitate was dried in *vacuo*.

The dried precipitate was dissolved in a small volume of dilute ethanol, cooled for 30 minutes and centrifuged. The undissolved residue was rejected. The supernatant liquid was precipitated with double the volume of 95 to 96 per cent ethanol, left at the room temperature for 15 minutes and then centrifuged. The precipitate rich in bound nicotinic acid was dried *in vacuo* and the clear liquid at the top rejected. This precipitate was again dissolved in a small volume of dilute ethanol and centrifuged. The residue was discarded. The supernatant liquid was then precipitated with equal volume of 95 to 96 per cent ethanol. The turbid liquid was immediately decanted off and the precipitate which was sticking to the sides of the tube was dried *in vacuo*. This precipitate which was brownish in appearance contained about 20 mg. of nicotinic acid per gramme of the material. It had been ascertained by the method of Chaudhuri (*Science and Culture*, 1951, **17**, p. 270) that all the nicotinic acid in the final precipitate so obtained was bound up with the other moiety of the unknown compound. This purified compound gave an acidic reaction in solution. It also gave some of the important protein tests. It is, therefore, possible that the nicotinic acid may be linked up through its carboxylic group with the amino group of the polypeptide leaving its acidic groups predominantly in the free state.

Assuming that one mole of nicotinic acid is bound up with the rest of the molecule, it is possible to calculate theoretically the molecular weight of this purified compound taking into consideration that 20 mg. of nicotinic acid was present in one gramme of the purified material. On that basis the molecular weight would be 6,150.

The purified material obtained by the above procedure was then subjected to the process of column chromatography using permutit as adsorbent. A solution of the purified material containing 10 mg. per c.c. was prepared in dilute ethanol and passed through a column of permutit under water suction. The filtrate which appeared turbid was centrifuged and dried at 70° C. under reduced pressure. The dried residue was tested for bound nicotinic acid but it was found that no further purification had been attained in this fraction. The column was then eluted with a small amount of dilute hydrochloric acid. The eluate which was also slightly turbid was centrifuged and the clear liquid at the top dried at 70° C. under reduced pressure. This dried residue which also contained bound nicotinic acid indicated that no more purification of this unknown substance by this method was possible.

METABOLISM OF IRON DURING THE EMBRYONIC DEVELOPMENT OF HEN'S EGG.

Last year relative changes in the ionizable (non-hæmin) and non-ionizable (hæmin) iron content of hen's egg at different stages of embryonic development were reported on. The development of hæmin iron at the cost of non-hæmin iron proceeded at a slow rate till about the seventh day of incubation. From the seventh day onward the development was rapid and on the 20th day of incubation, just one day before hatching takes place about 75 per cent of the total iron was found to be present as hæmin iron. The whole work has been repeated and confirmed.

Since the character of hæmin molecule is greatly modified by its combination with the protein moiety, globin, which has the effect of making hæmin more soluble and making its reaction with oxygen a reversible one,

it was thought desirable to follow up the relative changes in the ionizable (non-hæmin) and non-ionizable (hæmin) iron in relation to the oxygen-absorption capacity of the hæmoglobin molecule at the different stages of development of the chick embryo. The oxygen-absorption capacity of blood from the developing chicken embryo was measured by the manometric gas-analysis method of van Slyke (*Jour. Biol. Chem.*, 1924, **61**, p. 523; *ibid.*, 1927, **73**, p. 121). The blood of the chicken embryo was drawn from the umbilical cord by an oxalated hypodermic syringe. The blood thus obtained was diluted with physiological saline so that no hæmolysis occurs before the gas analysis. Generally one c.c. of diluted blood was taken for the analysis. It was observed that the volume per cent oxygen capacity of the blood from the chick embryo on the tenth day of incubation was about four to five and the value rose steadily up to ten on the 15th day. On the 19th day of incubation the volume per cent oxygen capacity was about 19. The blood was obtained by cardiac puncture on the day of hatching and the volume per cent oxygen capacity varied from 19 to 21 depending on the development of the young chick. After hatching the oxygen-absorption capacity falls to nearly 16.

The oxygen-absorption capacity of blood represents the volume of oxygen required to combine with all of the hæmoglobin in 100 c.c. of blood. It, therefore, constitutes a measure of hæmoglobin since it is known that each c.c. of oxygen absorbed represents 0.736 g. of hæmoglobin in an adult animal. Also one g. of hæmoglobin contains 0.0034 g. of iron. So the amount of iron was determined in the blood (*a*) from the oxygen-absorption capacity and (*b*) also by the chemical method according to Ramsay (*Biochem. Jour.*, 1951, **49**, p. 494) during the different stages of embryonic development. The values for iron obtained by method (*a*) are higher than those obtained by method (*b*) in the earlier stages of embryonic development. They become equal after hatching. This indicates that the hæmoglobin in the earlier stages has a higher degree of affinity for oxygen than when the chick has hatched. The work is in progress.

EFFECT OF CANNING AND STORAGE ON THE NUTRITIVE VALUES OF SOME COMMON INDIAN VEGETABLES.

Cabbage (*Brassica oleraceacapitata*). It was reported last year that ascorbic acid was destroyed severely in cabbage during canning operations. Thermal treatments which are necessary for canning may cause appreciable destruction of nutrients, particularly of ascorbic acid, when such treatments are carried out in presence of oxygen. Experiments were designed to study the effect of blanching, holding the blanched samples exposed to air, both hot and cooled, cooking (autoclaving) and storage at room temperature and also the fate of added synthetic ascorbic acid to the samples of cabbage. The canning operations were carried out at the Department of Food Technology, College of Engineering & Technology, Jadavpur, West Bengal.

Cabbage was purchased from the local market. Ten to twelve pounds of the samples were brought to the laboratory, washed in tap water, dried with towel and then shredded. The shredded samples were mixed thoroughly and four representative samples were taken to determine the

ascorbic-acid content. The method for estimation of ascorbic acid was that recommended by the Association of Vitamin Chemists, Inc. (1947).

The samples of cabbage were blanched for four minutes at 82.2° C. with about ten volumes of water. After blanching, a portion was cooled under tap and the remaining portion was kept as it is. Moisture and ascorbic-acid content of the samples were determined at intervals of 5, 30 and 60 minutes.

One hundred grammes of blanched samples were put into cans of the size 301 × 309, covered with 1.5 per cent sodium-chloride solution, leaving a head space of one-eighth inch. Filled cans were exhausted by placing in a water-bath till the temperature of the contents of the cans reached 70° C. They were then sealed by double seaming. The sealed cans were cooked (autoclaved) at 10-lb. pressure for 30 minutes, cooled to room temperature by a spray of water and stored in the Laboratory.

One can was opened immediately after canning and others after seven days, 15 days, one month, two months and three months, respectively. They were drained for five minutes in a large colander. Weights of solid portions and volumes of liquid portions were recorded. The ascorbic-acid contents of both the solid and liquid portions were determined and the value of total can contents was calculated.

Effect of blanching and exposure to air of the blanched samples, (a) hot and (b) cooled: The blanched samples of cabbage were divided into two portions. One portion was cooled under tap and the other portion kept as it is. Both the hot and cooled portions were exposed to the air in a thin layer. The ascorbic-acid contents of six samples were estimated at intervals of five minutes, 30 minutes and 60 minutes. The total loss of ascorbic acid at different stages is tabulated below :—

Sample	Loss of ascorbic acid (on dry basis), per cent		
	5 minutes	30 minutes	60 minutes
Cooled ...	18.0	20.5	28.2
Hot ...	20.5	25.6	30.7

It will appear from the above data that blanched and hot samples lose somewhat more ascorbic acid than blanched and cooled samples. The results also show that the longer the time of holding the blanched samples exposed to air the greater the destruction of ascorbic acid.

Effect of cooking (autoclaving) at 10-lb. pressure for 30 minutes: The most severe destruction of ascorbic acid of the samples occurred during cooking or autoclaving. The average total loss up to the end of cooking operation comes to about 92 per cent. Of the remaining vitamin in the can about 39 per cent is found in the solid portion and 61 per cent in brine.

Effect of storage of the canned samples at room temperature: The canned samples were stored in the Laboratory at room temperature. One can from each lot was opened after seven days, 15 days, one month, two months and three months, respectively. The total loss of ascorbic acid up to the end of 7 days and 15 days are 94.0 per cent and 94.4 per cent, respectively,

after which there seems to be practically no further loss in the whole can. Of the retained ascorbic acid in the can approximately 37.0 per cent is found in the solid portion and 63.0 per cent in brine.

Fate of added synthetic ascorbic acid in the can : One hundred mg. synthetic ascorbic acid were added to the contents of the cans just before sealing. One can from each lot of samples was opened immediately after cooking and the ascorbic-acid content of the solid and liquid portions was determined. The effect of storage at room temperature was also studied. It was found that about 36.0 per cent of the total ascorbic acid in the can was destroyed during cooking. Up to the end of 7 days, 15 days, one month, two months and three months, the total loss of ascorbic acid in the whole can came to 45.0 per cent, 52.0 per cent, 52.2, 54.1 and 54.5, respectively. In other words, after cooking 64.0 per cent of ascorbic acid was found in the total can contents and during storage the average retention value was 55.0, 48.0, 47.8, 46.0 and 45.0 per cent after 7 days, 15 days, one month, two months and three months, respectively. The distribution of the retained ascorbic acid in the can between cabbage and brine was found to be more or less constant during the whole period of storage, about 25 per cent in the solid portion and 75 per cent in brine.

Organoleptic changes : As observed last year there was no further appreciable damage in organoleptic ratings during storage. The change was appreciable during cooking (autoclaving) only. The inclusion of added synthetic ascorbic acid seems to have no effect on organoleptic changes.

Ascorbic-acid content of canned cabbage obtained from the manufacturers in India : Two lots of samples were received from the manufacturers in India. Lot No. 1 contained on the average 3.2 mg. of ascorbic acid per 100 mg. solids on wet basis (moisture content 91.0 per cent) and 3.9 mg. of ascorbic acid per 100 c.c. of brine. Lot No. 2 had on the average 0.37 mg. of ascorbic acid per 100 g. solid on wet basis (moisture content 92 per cent) and 1.2 mg./100 c.c. brine.

Ascorbic-acid content of canned cabbage obtained from the market : Only one brand of canned cabbage was available in the local market. The sample was the same as lot No. 2 obtained from the manufacturer and the ascorbic-acid contents of solid and brine were the same.

2. *Cauliflower* (*Brassica oleracea botrytes*) : It was reported last year that there was no loss of ascorbic acid in cauliflower during blanching. This observation has been confirmed. Studies on the loss of ascorbic acid during cooking (autoclaving) were done. Experimental procedures were similar to those in cabbage. It has been observed that about 40 per cent of ascorbic acid of the blanched samples is destroyed during cooking. Of the remaining, about 30 per cent is found in the solid portion and 70 per cent in brine. Studies on the retention of ascorbic acid in cauliflower during operations in canning are in progress on lines as outlined in the case of cabbage.

3. *Parwar* (*Coccinia indica*) : Detailed studies on the retention of ascorbic acid in parwar during different stages of canning and storage have been undertaken. Changes in thiamin, riboflavin, nicotinic-acid, calcium phosphorus and iron contents at different stages of canning operations are also being studied.

THIAMIN VALUES OF PURE-BRED STRAINS OF CEREALS AND PULSES.

Studies on the thiamin content of pure-bred strains of cereals and pulses are being continued. After the submission of the last report about 105 more samples received in 1952-53 were analysed. Thus, the total number of samples analysed comes to 175. The results are given in Table IV:—

TABLE IV.

Thiamin content of cereals and pulses.

Rice samples.

Received from		Strain number	Thiamin content μ ./g.m.
Ambasamudram	...	ASD 1	1.4
"	...	ASD 4	2.0
"	...	ASD 5	3.0
Pattambi	...	Ptb 2	2.8
"	...	Ptb 4	2.2
"	...	Ptb 10	2.2
"	...	Ptb 15	3.3
"	...	Ptb 22	1.9
Aduturai	...	ADT 1	3.3
"	...	ADT 2	2.5
"	...	ADT 3	4.5
"	...	ADT 20	5.0
Maruteru	...	ADT 23	2.6
"	...	MTU 1	4.1
"	...	MTU 3	4.5
"	...	MTU 10	3.9
"	...	MTU 15	4.7
"	...	MTU 19	3.4
"	...	BC 130	1.7
Coimbatore	...	CO 13	1.8
"	...	CO 25	2.1
"	...	CO 19	2.6
"	...	CO 20	2.5
"	...	CO 34	2.0
"	...	GEB 24	1.4
Samalkot	...	SLO 2	4.7
"	...	SLO 13	4.4
I.A.R.I., Pusa	...	NP 31	2.8
"	...	NP 52	3.0
C.R.R.I., Cuttack	...	CH 45	3.2
"	"	GEB 24	3.9
"	"	T 90	3.9
"	"	CO 13	4.2
"	"	T 1145	3.6
"	"	PTB 10	2.1
"	"	T 141	3.6

TABLE IV—(contd.)

Received from	Strain number	Thiamin content $\mu\text{g./g.}$
C.R.R.I., Cuttack	BAM 6	2.7
" "	CO 16	3.6
" "	CH 47	3.2
" "	T 1242	3.0
Chinsurah, West Bengal	Charnock	5.2
" "	Bhutmuri	4.4
" "	Dharial	4.0
" "	Kaimurali	2.7
" "	Badsa bhog	4.6
" "	Bhasamanik	3.3
" "	Orissa kankuria	3.5
" "	Askata	4.0
" "	Assam Boro	3.5
" "	Tilak kachari	3.3
" "	Nagra	3.9
" "	Jhanji 34	3.5
Buchireddipalem	BCP 1	3.3
Nagina, U.P.	T 12	2.1
"	T 25	3.0
"	T 36	2.9
"	T 21	2.5
"	T 15	3.5
"	T 9	3.9
"	A 64	2.2
"	A 17	2.2
"	T 3	3.2
"	T 56	2.8
"	CH 4	2.9
"	T 112	2.2
"	Anjana Haldwani	2.9
"	T 137	2.3
"	T 119	2.3
"	T 23	2.6
"	T 1	2.5
"	CH 10	2.3
"	T 127	1.9
"	T 88	2.2
"	N 32	3.8
"	T 26	5.4
"	T 15	4.1
"	T 5	4.1
"	N 22	2.0
"	N 27	1.9
"	T 43	1.5
"	T 29	1.0
"	N 10 B	2.0
"	N 12	1.9
"	T 110	2.5
"	T 136	1.7

TABLE IV—(contd.)

Other samples.

Received from			Strain number	Thiamin content μg./g.m.
Hyderabad	Tur C 11	3.7
"	Tur C 28	2.9
"	Tur C 36	3.8
Vizianagram	Green gram 127	2.9
"	Red gram 97	2.8
"	Black gram 189	1.9
Coimbatore	Green gram 62	2.3
"	Red gram 37	2.9
"	Black gram 212	3.0
"	Bengal gram 482	2.6
Bellary	Sorghum H 1	2.6
"	Setaria H 1	3.9
"	" H 2	4.1
Nandyal	Sorghum N 1	1.1
"	" N 4	1.5
"	Setaria N 1	3.1
Coimbatore	Sorghum CO 1	1.8
"	" CO 7	1.5
"	" CO 9	1.8
"	" CO 12	1.5
"	Bajra CO 1	1.8
"	" CO 3	2.4
"	Stzearia CO 1	3.9
"	" CO 3	4.0
"	Ragi CO 2	0.8
Kanpur	Wheat 720	5.0
"	" C 13	3.5
"	" NP 710	4.3
"	" NP 775	4.5
"	" NP 52	4.0
"	" NP 125	4.3
"	" PB 591	5.0
"	" NP 770	3.0
"	" C 591	2.0
"	" C 518	2.7
"	" K 13	3.0
"	Gram T 87	0.8
"	Arhar 17 W/2	2.3
"	Mung T 1	2.0
"	Urid T 27	2.4
I.A.R.I., Pusa	" NP 4	1.3
"	"	...	" NP 6	1.4
"	"	...	" NP 7	1.9
"	"	...	" NP 14	1.4
"	"	...	Arhar NP 24	3.0

The samples were obtained as whole grains. All the pulses and cereals except paddy were analysed for their thiamin content without removing the outer husk. The paddy samples were, however, first shelled by a P.S.G. sheller at a standard speed of 90 strokes per minute for one minute and the rice was taken for analysis. For the estimation the method of Harris and Wang (*Biochem. Jour.*, 1941, **35**, p. 1050) was followed.

TABLE IV—(contd.)

Received from	Strain number	Thiamin content $\mu\text{g./g.m.}$
I.A.R.I., Pusa ...	Arhar NP 31	1.6
" " ...	" NP 69	0.5
" " ...	" NP 80	4.2
" " ...	Mung NP 28	1.4
" " ...	" NP 36	0.4
" " ...	" NP 18	2.4
" " ...	" NP 23	1.8
" " ...	Lentil NPH 1	1.8
" " ...	" NP 11	2.0
" " ...	Wheat NP 710	4.0
" " ...	" NP 715	3.3
" " ...	" NP 755	4.5
" " ...	" NP 762	4.2
" " ...	" NP 4	5.2
" " ...	" NP 775	4.6
" " ...	" NP 737	3.9
" " ...	" NP 760	4.1
" " ...	" NP 761	4.4
" " ...	" NP 764	3.6
" " ...	" NP 52	5.7
" " ...	" NP 720	4.1
" " ...	" MH 43	5.0
" " ...	" PC 36	3.9
" " ...	" NP 12	6.7
" " ...	" NP 745	5.4
" " ...	" NP 165	4.6
" " ...	" NP 768	4.0
" " ...	" NP 111	5.1
" " ...	" NP 235	4.1
" " ...	" NP 24	2.7
Chinsurah, West Bengal ...	" NP 4	4.4
" " " ...	" NP 52	4.4
" " " ...	" NP 12	4.4
" " " ...	" Pepsu	4.1
" " " ...	" Jamali (local)	5.0
" " " ...	" M.P. 111	3.7
" " " ...	" MP 165	3.3
" " " ...	Lentil	2.8
" " " ...	Khesari 13	4.1
" " " ...	Arhar 62	4.5
" " " ...	" 22	1.7
" " " ...	Gram S-4	2.0

The work is being continued this year on the same varieties but as obtained from the succeeding year's crop. The results of the samples analysed this year so far and those of the last year's are given in Table V. It will be observed that the thiamin values of the samples, except in one or two, are in good agreement, although they are derived from two successive years' crops.

TABLE V.
Thiamin values of cereals and pulses.

Received from	Strain number	Thiamin content $\mu\text{g./g.}$	
		1952-53	1953-54
Nagina, U.P.	Rice T 12	2.1	2.2
"	" T 25	3.0	3.3
"	" T 36	2.9	3.2
"	" T 21	2.5	2.5
"	" T 15	3.5	3.8
"	" T 9	3.9	3.5
"	" A 64	2.2	2.5
"	" T 17	2.2	2.5
"	" T 3	3.2	6.0
"	" T 56	2.8	3.0
"	" CH 4	2.9	3.1
"	" T 112	3.2	3.7
"	" Anjana	2.9	2.5
"	" T 137	2.3	2.5
"	" T 119	2.3	2.3
"	" T 23	3.6	4.0
"	" T 1	2.5	2.7
"	" CH 10	2.3	2.5
"	" T 127	1.9	1.6
"	" T 88	2.2	2.4
"	" N 32	3.8	3.8
"	" T 26	5.4	5.3
"	" T 105	4.1	4.0
"	" T 5	4.1	3.9
"	" N 22	2.0	2.0
"	" N 27	1.9	1.9
"	" T 43	1.5	4.1
"	" T 29	1.0	1.1
"	" N 10 B	2.0	2.2
"	" N 12	1.9	2.0
"	" T 110	2.5	2.7
"	" T 136	1.7	1.5
Buchireddipalem	" BCP 1	3.3	3.4
Ambasamudram	" ASD 1	1.4	1.5
"	" ASD 4	2.0	2.3
"	" ASD 5	3.0	3.2
Nandyal	Sorghum N 1	1.1	1.0
"	Sorghum N 4	1.5	1.0
"	Setaria N 1	3.1	3.1
Bellary	Sorghum H 1	2.6	2.5
"	Setaria H 1	3.9	3.5
"	" H 2	4.1	3.3

TABLE V.—(contd.)

Received from	Strain number	Thiamin content $\mu\text{g./g.}$	
		1952-53	1953-54
Hyderabad ...	Tur C 11	3.7	3.4
„ ...	„ C 28	2.9	3.1
„ ...	„ C 36	3.8	3.8

The rice samples were analysed according to the method of Harris and Wang (*loc. cit.*) and by the method described by the Medical Research Council (*Biochem. Jour.*, 1943, **37**, p. 433). The lower recovery of added thiamin in rice samples analysed by the Med. Res. Coun. method was observed in the estimations of this year also.

From the results of analyses by the two methods it appears that in the *Med. Res. Coun.* method, the added vitamin is lost to the extent of about 30 to 40 per cent. To elucidate this point it was felt necessary to know if rice has got any thiamin destroying factor. Preliminary experiments show that some rice samples in which the recovery of added thaimin is very poor when estimated by the *Med. Res. Coun.* method do possess thiamin-destroying factor. Further studies are in progress.

3. Nutrition research unit under Dr. R. G. Chitre at Seth G. S. Medical College, Bombay.

During the year under report the work on the nutritive value of pure-bred strains of cereals and pulses was continued. The estimations of eight amino acids by chemical and microbiological methods were completed. As regards estimations of vitamins of B-group, few representative samples of pure-bred strains of cereals and pulses were again analysed for their thiamine, riboflavin and nicotinic-acid contents after a period of six months' storage. This part of the study was incidental and is reported only because of interesting results. The microbiological assay of folic-acid contents from these strains as well as its physiological availability is newly undertaken.

This year the work on the problems : (1) the biosynthesis of nicotinic acid in germinating cereals and the rôle of alkali-labile precursor in such a synthesis ; and (2) the physiological availability of essential nutrients—which was held in abeyance—was re-started. The effects of certain known nicotinic-acid precursors in animal organism were studied on germinating cereals.

One more noteworthy feature during the year is that the Unit could undertake investigation to throw light on the interrelation between nutrition and kidney function in human subjects. The influence of protein nutrition on 'urea clearance' values in humans has been carefully investigated. The results will be found interesting.

Following are the details of individual investigation:—

(1) *Nutritive value of pure bred-strains of cereals and pulses.*

(a) *Estimations of amino acids.*—Few representative samples of pure-bred strains of cereals and pulses were analysed for their arginine, histidine, methionine, cystine, phenyl-alanine, leucine, valine and threonine contents. The first four amino acids were estimated by chemical methods and the rest by microbiological methods. The results are shown in Tables I and II. Further work is in progress.

TABLE I.
Chemical estimations of amino acids.
(Figures are in g. per cent).

Serial number	Strain number	Methionine	Cystine	Histidine	Arginine
I. RICE (<i>Oryza sativa</i>).					
<i>Aduturai</i>					
5	ADT-23	0·298	0·249	0·331	1·302
<i>Pattambi</i>					
6	PTB-10	0·298	0·249	0·381	1·172
7	PTB-22	0·301	0·249	0·309	1·172
8	PTB-2	0·258	0·249	0·286	1·172
9	PTB-4	0·337	0·305	0·286	1·302
10	PTB-15	0·232	0·277	0·286	1·042
<i>Samalkot</i>					
11	SLO-2	0·302	0·249	0·331	0·810
12	SLO-13	0·270	0·305	0·357	0·984
<i>Maruturai</i>					
47	BC-130	0·209	0·222	0·357	1·042
50	MTU-1	0·371	0·222	0·357	0·984
<i>Cuttuck</i>					
51	T.608	0·394	0·249	0·405	1·042
54	FR 13A	0·302	0·194	0·357	0·984
55	C.H. 47	0·371	0·194	0·381	0·984
56	PTB-10	0·384	0·222	0·452	0·984
58	CO-13	0·394	0·278	0·357	1·158
59	NO. 136	0·255	0·222	0·452	1·100
60	CH. 45	0·302	0·278	0·428	1·100
61	Benibaog	0·255	0·249	0·405	1·042
62	T. 1145	0·278	0·221	0·380	0·892
*64	BAM-6	0·278	0·222	0·405	1·274
*66	CO-16	0·302	0·194	0·428	0·926
*67	CH.2	0·302	0·166	0·381	1·042
68	T-90	0·325	0·166	0·257	0·926
69	BAM. 9	0·302	0·194	0·381	0·869
70	T. 412	0·278	0·166	0·418	1·042
<i>Coimbatore</i>					
71	CO. 3	0·325	0·222	0·357	1·042
73	CO. 19	0·278	0·249	0·418	0·984
74	CO. 20	0·348	0·222	0·452	1·216
75	GEB. 24	0·348	0·222	0·381	1·042
76	CO. 25	0·241	0·116	0·357	0·926
II. JOWAR (<i>Sorghum vulgare</i>).					
<i>Nandyal</i>					
17	N-1	0·394	0·167	0·355	0·781
<i>Coimbatore</i>					
19	CO-7	0·278	0·208	0·437	0·911
<i>Bellary</i>					
32	H-1	0·321	...	0·270	0·797

TABLE I—(contd.)

Serial number	Strain number	Methionine	Cystine	Histidine	Arginine
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III. BAJRI. (*Pennisetum typhoideum*).

Coimbatore					
23	CO-1	0.325	...	0.458	0.911
24	CO-3	0.348	0.404	0.458	1.042

IV. RAGI (*Eleusine coracana*).

Coimbatore					
25	CO-2	0.325	0.260	0.233	0.637

V. TENAI (*Setaria italica*).

Nandyal					
26	N-1	0.348	0.260	0.370	0.736
Coimbatore					
27	CO-1	0.394	0.260	0.354	0.858
28	CO-3	0.302	0.258	0.528	1.25
Bellary					
33	H-2	0.394	0.333	0.473	0.797
34	H-1	0.209	0.364	0.222	0.797

VI. RED GRAM (*Cajanus indicus*).

Himayat sagar					
29	C-11	1.499	1.14	0.912	0.693
30	C-28	0.952	0.463	...	2.776
31	C-36	0.417	0.509	...	3.331
Vizianagaram					
35	NO. 97	1.578	1.296	0.701	0.659
Coimbatore					
43	No. 37	1.730	1.344	0.948	0.674

VII. GREEN GRAM (*Phascaus radiatus*).

Vizianagaram					
36	No. 127	0.852	1.134	1.100	0.741
Coimbatore					
44	No. 62	0.648	0.324	0.692	2.776

VIII. HORSE GRAM (*Dolichos biforus*).

Vizianagaram					
37	No. 93	0.556	0.508	1.042	3.274
39	No. 76	0.463	0.508	1.375	3.274
Coimbatore					
40	No.D.B.7	0.417	0.508	1.208	3.125

TABLE I—(contd.)

Serial number	Strain number	Methionine	Cystine	Histidine	Arginine
IX. BLACK GRAM (<i>Phaseolus mungo</i>).					
<i>Vizianagaram</i> 38	No. 189	0·741	0·417	1·855	3·125
<i>Coimbatore</i> 42	No. 212	0·787	0·370	1·458	3·331
<i>Kanpur</i> 90	NP. 4	0·556	0·365	1·003	2·818
X. BENGAL GRAM (<i>Cicer arietinum</i>).					
<i>Coimbatore</i> 41	No. 482	0·695	0·370	1·100	5·570

TABLE II.
Microbiological estimations of amino acids.
(Figures are in g. per cent).

Serial number	Strain number	Phenylalanine	Leucine	Valine	Threonine
I. RICE (<i>Oryza sativa</i>).					
<i>Aduturai</i> 1	ADT-1	0·463	0·779	0·692	0·433
<i>Pattambi</i> 8	PTB-2	0·456	0·916	0·608	0·379
<i>Samalkot</i> 11	SLO-2	0·573	1·034	0·520	0·322
<i>Ambasamudram</i> 14	ASD-4	0·498	1·030	0·722	0·459
<i>Maruterai</i> 47	BC 130	0·441	0·821	0·662	0·422
50	MTU-1	0·496	0·667	0·629	0·422
<i>Cuttuck</i> 54	F.R.13A	0·452	0·667	0·583	0·357
58	CO-13	0·394	0·755	0·595	0·406
70	T.412	0·426	0·734	0·574	0·368
<i>Pusa</i> 82	N.P. 31	0·401	0·866	0·637	0·313
II. JOWAR (<i>Sorghum vulgare</i>).					
<i>Nandyal</i> 17	N-1	0·678	1·250	0·600	0·361
<i>Coimbatore</i> 20	CO-7	0·447	1·450	0·686	0·357
<i>Bellary</i> 32	H-1	0·533	1·208	0·548	0·348

TABLE II—(contd.)

Serial number	Strain	Phenylalanine	Leucine	Valine	Threonine
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V. TENAI (*Setaria italica*).

Nandyal					
26	N-1	0.548	1.358	0.618	0.888
Bellary					
33	H-2	0.582	1.653	0.656	0.361

VI. RED GRAM (*Cajanus indicus*).

Himayat sagar					
29	C-11	1.499	1.14	0.912	0.693
Vizianagaram					
35	No. 97	1.578	1.296	0.701	0.659
Coimbatore					
43	No. 37	1.730	1.344	0.948	0.674

VII. GREEN GRAM (*Phaseolus radiatus*).

Vizianagaram					
36	No. 127	0.852	1.934	1.100	0.741
Kanpur					
79	T. 1	0.964	1.664	1.048	0.614

VIII. HORSE GRAM (*Dolichos biflorus*).

Vizianagaram					
39	NO. 76	1.000	2.218

IX. BLACK GRAM (*Phaseolus mungo*).

Kanpur					
77	T. 27	1.205	1.552
Pusa					
89	N.P. 6	1.061	1.460

X. BENGAL GRAM (*Cicer arietinum*).

Kanpur					
80	T. 87	0.720	1.076	0.856	0.502

XI. WHEAT (*Triticum vulgare*).

Kanpur					
101	K-13	0.396	0.797	0.435	0.341
104	C-13	0.408	0.727	0.402	0.402
107	NP-125	0.364	0.681	0.396	0.366
109	NP-775	0.530	0.943	0.451	0.378

(b) *Estimation of thiamine, riboflavin and nicotinic-acid contents in some pure-bred strains of cereals and pulses on storage.*—Last year the results of analyses of thiamine, riboflavin and nicotinic-acid contents of pure-bred strains of cereals and pulses were reported. These strains were stored, some in gunny bags and some in sealed bottles. In view of the reports of Vinaeke (*Jour. Home Econ.*, **43**, p. 641, 1951); and Dayrit *et al.* (*Acta Medl. Philippina*, **6**, p. 113, 1949); and Speirs *et al.* (*Georgia and North Carolina Arg. Exp. Stations, Southern Coop. Ser. Bull.*, **3**, p. 5, 1945) on the effect of storage on vitamin contents, it was thought interesting to note whether any significant changes have taken place in the strains, as regards these vitamins. Samples of rice from Cuttuck and Coimbatore centres were, therefore, analysed for the vitamin contents after the storage of six months. The results are summarized in Table III :—

TABLE III.

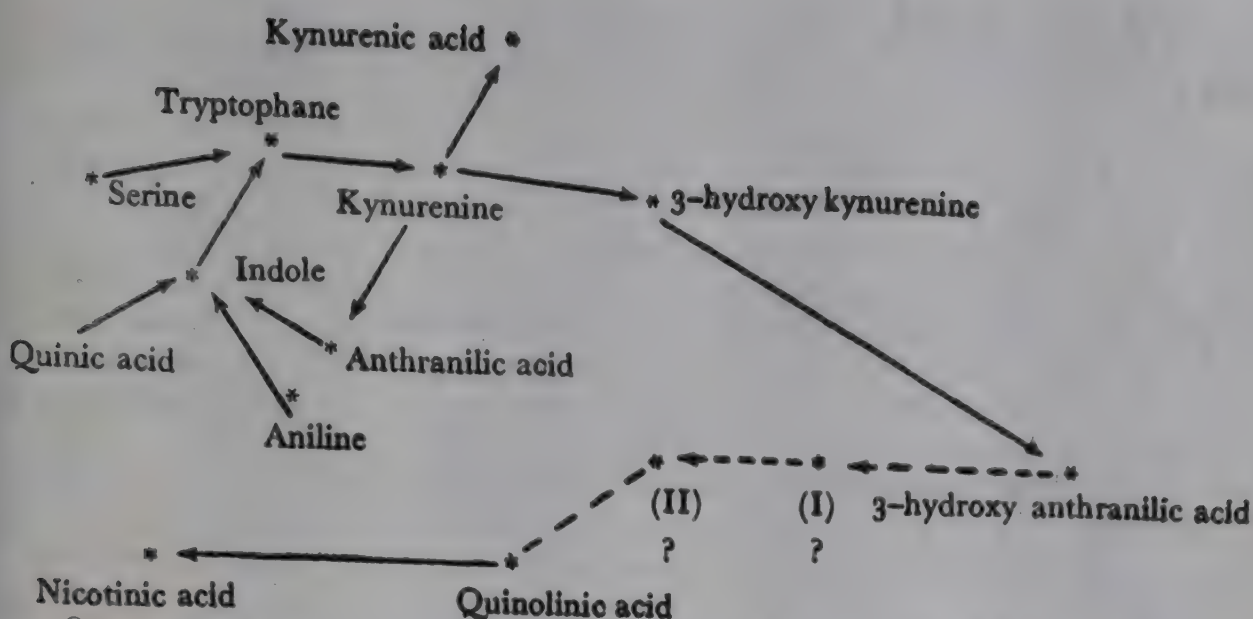
Mean thiamine, riboflavin and nicotinic acid contents of rice samples after the storage for six months.

	Initial	Stored in sealed bottles	Stored in gunny bags
Thiamine (μ g./100 g. ...)	273.5 \pm 16.17	272.1 \pm 15.23	164.2 \pm 8.96
Riboflavin (μ g./100 g.) ...	54.0 \pm 9.45	82.7 \pm 6.48	105.0 \pm 8.92
Nicotinic acid (mg./100 g.)	2.44 \pm 0.153	2.47 \pm 0.087	2.34 \pm 0.182

The results point out, that during six months' storage in sealed bottles there was no appreciable loss either in the thiamine or the nicotinic-acid contents of rice. In the case of riboflavin, however, there was a noticeable increase. The storage for the same period in gunny bags gave different results. There was appreciable loss in the thiamine content and much more increase in the riboflavin content. There was no change in the nicotinic-acid contents. The changes are the reflections of metabolic reactions in the grains on storage. Greater changes during the storage in gunny bags could be understood in view of the fact that the seeds under those conditions have more access to atmospheric oxygen. This might eventually accelerate some metabolic processes within the seeds. From the nutritional point of view such studies would help to improve storage conditions suitable to our climatic conditions. In view of the above interesting results the work is being extended to other types of strains and also for longer periods of storage.

(2) *Biosynthesis of nicotinic acid in germinating cereals and pulses.*

(a) *The effect of quinolinic and kynurenic acids on the biosynthesis of nicotinic acid in germinating cereals and pulses.*—The present status of knowledge of this intricate problem based on studies in several species could be summarized as follows :—



It has been shown earlier (*Technical Report, I.C.M.R.*, p. 57, 1951) that tryptophane acts as a precursor of nicotinic acid in germinating cereals and pulses. However, the actual intermediates of this conversion are not known. From what has been shown in the case of animals and certain species of neurospora it is likely that the same or similar intermediates might exist.

Many of these intermediates are not easily available in this country. The work was, therefore, held in abeyance last year. Dr. L. M. Henderson of the University of Illinois has kindly supplied some of those intermediates. As such the effect of quinolinic and kynurenic acids was studied on the biosynthesis of nicotinic acid in germinating cereals and pulses. The work on three pulses has been completed. The results so far obtained have shown that kynurenic acid had no effect, while quinolinic acid in the germinating fluid increased the nicotinic acid biosynthesised by germinating pulses. The work is being confirmed. The effect of other intermediates is also being studied.

(b) *Rôle of alkali-labile precursor in the biosynthesis of nicotinic acid during germination.*—It has been reported earlier (*Technical Report, I.C.M.R.*, p. 57, 1951) that the alkali-labile precursor is decreased in the germinating cereals when there was added tryptophane in the germinating media with the simultaneous increase in the nicotinic acid, and that no such change occurred in the precursor when germination was carried in distilled water. This would suggest that probably the so-called alkali-labile precursor is not an intermediate in the chain of biosynthesis of nicotinic acid. It is interesting, therefore, to see what rôle it performs in the seed. It might, perhaps, be an accumulated product of nicotinic-acid metabolism at some stage under some specific conditions. This aspect of the problem could be understood by systematic observations of the changes in the nicotinic-acid metabolites at various stages of growth of the seed on the plant. The work in this direction has just started and nicotinic-acid metabolites in rice, during various stages of the growth of paddy, are being studied. Preliminary results have shown that the precursor appears in the paddy just before it is ready for harvesting, while the nicotinic acid is present long before. More interesting results are awaited.

(3) *Physiological availability of essential nutrients : Folic acid.*

Thiamine, riboflavin and nicotinic-acid contents of pure-bred strains of cereals have been reported and the physiological availability *in vivo*

To start with one hundred clinically normal subjects—mostly medical staff and students between the ages 20 and 38 years—were studied for their 'urea clearance' by van Slyke's technique. Out of these, 47 subjects subsisted on mixed dietary, while 53 were strictly vegeterians. Table VI shows the results of the study :—

TABLE VI.

'Urea-clearance' values in normal human subjects.

Number of subjects	Urea clearance	Range	Mean and standard deviation
58	Maximum	40–59·5	$45·6 \pm 3·64$
42	Standard	31·5–47·0	$36·6 \pm 2·80$

These figures once more showed that the urea clearance values are low in normal Indians as compared with the Westerners.

The next thing to see was whether these low clearance values were related with the low protein nutrition of the subjects. For the purpose, 20 clinically normal subjects were selected. They were between the ages 20 and 38 years. Majority were from the medical students and doctors who knew the implications of the investigation and co-operated very well. Out of these, ten were subsisting on mixed diet and their protein intake varied from 41·75 g. to 65·75 g. per day with an average 47·7 g. The remaining ten were strictly vegeterians and their protein intake varied from 32 g. to 40 g. per day with an average of 37·25 g. Each of the subjects was studied for his 'urea clearance' on his routine dietary rigime for three times. Thereafter, the subjects were put on high protein diet of 80 g. to 84 g. per day for a period of one month. The enhancement of protein intake was from the sources similar to their basic dietary habits. After the lapse of the period, the subjects were re-examined for their 'urea-clearance' values. Finally, they were brought back on their basic dietary. Their 'urea-clearance' values were again investigated after a period of two months. The results of the investigation are summarized in Tables VII and VII-a which will be found interesting.

TABLE VII.

Mean 'urea-clearance' values in ten vegetarian subjects before and after high protein intake.

Diet	Standard clearance, c.c.	Maximum clearance and standard deviation, c.c.
(1) Basic dietary ...	$32·2 \pm 2·87$	$43·2 \pm 2·33$
(2) High protein diet (one month) ...	$55·6 \pm 3·29$	$76·6 \pm 3·001$
(3) Basic dietary (again after two months) ...	$34·8 \pm 2·9$	$41·7 \pm 2·50$

TABLE VII-a.

Mean 'urea-clearance' values in ten subjects on mixed dietary before and after high protein intake.

Diet	Standard clearance, c.c.	Maximum clearance and standard deviation, c.c.
(1) Basic dietary	38 \pm 4.88	44.1 \pm 1.14
(2) High protein diet (one month)	58.2 \pm 2.44	76.4 \pm 3.96
(3) Basic dietary (again after two months)	37.4 \pm 4.2	43.7 \pm 2.15

These results showed that the primary reason for the low 'urea-clearance' values in Indians, is low protein intake. Secondly, they also point out that the 'urea-clearance' values, do not depend on the type of protein consumed. Both vegetable and animal protein showed similar influence on the 'urea-clearance'.

The findings are of great significance from the view point of the clinical interpretations of kidney function in disease. Until adequate Indian standards are established for the purpose, clinicians should take into account the protein nutrition of the diseased subjects to assess the status of his kidney function based on the van Slyke's 'urea-clearance' test.

4. Inquiry on physiological effects of dietary protein under Dr. M. Damodaran at National Chemical Laboratory of India, Poona-8.

OBJECT OF THE INVESTIGATION.

The effect of feeding the three diets* A, B and C on the serum-protein components as studied by electrophoresis and the myoglobin content of the muscle are reported here. Nitrogen balance was studied in the rats at the end of 4th, 15th and 20th week and the rats were in positive nitrogen balance. At the end of five months on the diets, the animals were bled by heart puncture for the serum-protein analysis and sacrificed subsequently for the estimation of muscle hæmoglobin.

Electrophoretic analysis.—The electrophoretic analysis of the serum was carried out at 0°C. in the Perkin Elmer Tieselius apparatus, employing a 2-c.c. cell, veronal buffer of pH 8.6 with an ionic strength of 0.1 was used (Longworth, 1942). Serum samples were diluted with two parts of the

* The composition of the diets were :—

Group.	Rice flour.	Cane sugar.	Salt mixture.	Hydrogenated vegetable oil.	Protein supplement.
A	70	21	4	5	...
B	70	18	4	5	3 per cent pulse protein
C	70	6	4	5	15 per cent casein

buffer and dialysed against the same buffer for 72 hours at 0°C. prior to analysis. The final concentration of the protein solution was made approximately to 1.5 g. per cent. Photographs of the ascending and descending boundaries were obtained by the scanning method of Longworth. The photographs were enlarged three times and the area under each peak measured with a planimeter. Details of separation and measurement of the individual peaks were as described by Longworth. Table I gives the values for total serum protein ($N \times 6.25$) per 100 c.c. and its electrophoretic components. The concentration of albumin per 100 c.c. serum is seen to be markedly low in the rats maintained on the five per cent rice-protein diet compared to that of animals in group 'C'. On the other hand, there was no appreciable change in the globulin content of the serum.

Myoglobin content.—The extraction of myoglobin was done according to the procedure of de Duve (1948) employing N/100 acetate buffer and the separation and estimation by the method of Crandall and Drabkin, 1946. The pooled muscles from the hind and fore limbs were used. From the values given in Table II, it could be seen that at the end of five months on the diet, the myoglobin content became more than twice in the rats of the A and B groups, and three times in the controls, from an initial value of 0.1 mg./g. of muscle obtained after two weeks feeding on the diet. A high-protein diet is thus seen to favour an increase in the myoglobin content of the muscle.

TABLE I.

Electrophoretic concentrations of serum-protein components per 100 c.c. of serum.

'C' group.

Number of analysis	Serum protein, g./100 c.c.	Albumin, g./100 c.c.	Globulin, g./100 c.c.				
			Total []	α_1	α_2	β	γ
1	5.67	3.03	2.64	0.59	0.42	0.70	0.93
2	5.83	3.09	2.74	0.56	0.61	0.73	0.84
3	5.49	2.72	2.77	1.09	0.55	0.47	0.66
4	6.08	3.11	2.93	0.65	0.63	0.68	0.97
5	5.93	2.94	3.00	0.99	0.61	0.72	0.68
6	5.77	2.53	3.22	0.88	0.81	0.77	0.76
Average	5.80	2.90	2.88	0.79	0.60	0.68	0.81

'B' group.

1	5.18	2.37	2.78	0.69	0.49	0.80	0.80
2	4.67	2.21	2.45	0.76	0.46	0.58	0.65
3	4.95	2.52	2.43	0.68	0.52	0.58	0.65
4	5.54	2.83	2.71	0.55	0.62	0.71	0.82
5	5.93	2.16	2.78	0.70	0.58	0.73	0.77
6	5.18	2.65	2.52	0.83	0.49	0.64	0.56
7	5.41	2.74	2.69	0.69	0.61	0.67	0.72
Average	5.12	2.48	2.62	0.70	0.54	0.67	0.71

TABLE I—(contd.)

Number of analysis	Serum protein, g./100 c.c.	Albumin, g./100 c.c.	Globin—g./100 c.c.				
			Total [.]	α_1	α_2	β	γ
‘A’ group.							
1	5.36	2.05	3.25	0.84	0.81	0.73	0.87
2	4.13	1.86	2.28	0.58	0.50	0.54	0.66
3	4.44	2.06	2.38	0.68	0.49	0.57	0.64
4	4.41	2.17	2.24	0.60	0.48	0.52	0.64
5	4.55	1.94	2.62	0.81	0.53	0.64	0.64
6	4.53	1.96	2.57	0.88	0.52	0.61	0.56
7	3.73	1.35	2.39	1.04	0.40	0.51	0.44
Average	4.45	1.91	2.53	0.78	0.53	0.59	0.63

TABLE II.

Myoglobin content, mg./g. fresh muscle.

Number	Group A	Group B	Group C	Significance of difference between means
1	0.285	0.298	0.360	C <i>vs</i> A $t=4.3$
2	0.220	0.231	0.311	C <i>vs</i> B $t=3.4$
3	0.240	0.191	0.335	A <i>vs</i> B $t=.22$
4	0.260	0.299	0.312	
5	0.267	0.278	0.395	

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5. **Inquiry on metabolism of vitamin A under Dr. K. Rajagopal at the All-India Institute of Hygiene & Public Health, Calcutta.**

Vitamin A under normal conditions is only soluble in fats and fat solvents. In the visual cycle, vitamin A, in its aldehyde form, is in combination with a protein 'opsin' and functions in the water-soluble state. There is a reversible oxidation reduction between the alcohol and the aldehyde stages of vitamin A.

The functions of vitamin A are, however, not confined only to the visual system. It is required for the maintenance and regeneration of the cells of most of the organs of the animal body. Here also, it functions in the aqueous medium. Presence of vitamin A in water-soluble state was demonstrated by Cohn (1947) and Collins (1952) in plasma and cytoplasm.

For investigation of the functions of vitamin A in metabolism of the cell, the first requisite is to get vitamin A in a water-soluble state, quite homogeneous to the body fluids. This was achieved in this laboratory by combination of the vitamin A aldehyde with plasma albumin, separated by the elegant method of Cohn (1946), and was reported last year.

The biological activity of this compound—albumin-vitamin A aldehyde complex—has been thoroughly studied. Weanling rats and the mothers were fed on a diet with low amount of vitamin A. When these young rats weighed about 35 g. to 40 g. they were kept on a diet completely free from vitamin A. At the end of fifth week when their weights became stationary and on the point of decline, they were divided into three groups. One group of rats was fed with a supplement of vitamin A acetate and albumin, and the other with retinene-albumin complex, the third group was kept as control. The amounts of vitamin A alcohol (in acetate form) and vitamin A aldehyde (in combination with albumin), fed to each rat were kept almost equivalent; they were daily fed at a level of 35 I.U., with the help of specially devised micrometer-syringe with a ball pointed needle, in order to avoid any mechanical loss and to minimize the error of measurement. The liver storage of the vitamin was determined according to the method of March, English and Biely (1952) and the final estimation was done in a Beckman photo-electric spectrophotometer, adopting the modified irradiation technique of De (1937). The results are summarized in the Table:—

TABLE.

Experiment number	Number of rats in each group	Level of vitamin A (alcohol and aldehyde) intake, I.U.	Average increase in weight in each rat, g.	Level of vitamin A in liver/ g./I.U.
I	(a) 6	35	+27	4.5
	(b) 6	35	+24	3.8
	(c) 6 (4 died)	...	-35	Nil
II	(a) 8	35	+29	4.6
	(b) 8	35	+25	3.8
	(c) 7 (5 died)	...	-27	Nil
III	(a) 6	35	+26	4.4
	(b) 6	35	+25	3.3
	(c) 4 (3 died)	...	-30	Nil

(a) Albumin-retinene-fed group.

(b) Vitamin-A acetate albumin-fed group.

(c) Albumin-fed group.

From the results obtained it is evident that this water-soluble complex is better utilized than vitamin A acetate. A very recent work by Gounelle *et al.* (1952-53) also confirmed the findings of this Laboratory. These workers found that after administration of retinene, blood level of vitamin A rose to a considerable extent and no evidence of the presence of retinene in the blood could be found by the spectroscopic examination.

In order to investigate the physical nature of the aqueous solution of retinene-albumin complex, it was examined optically for any Tyndall-effect. Tyndall-effect in the visible region of spectra was found to be absent both in the albumin solution and in the retinene-albumin-complex solution. But another phenomenon became apparent. The retinene-albumin solution in phosphate buffer showed a beautiful bluish-green fluorescence, but it was found absent in albumin solution. From this finding it is apparent that this system of 'albumin-vitamin A aldehyde complex' forms with water more of the nature of a solution than a colloidal suspension.

The nature of combination between albumin and vitamin A aldehyde was first attempted to be investigated by the freezing-point method. Due to the presence of the potassium, phosphates and other inorganic ions in the solution, whose molecular weight were very low in comparison with that of albumin and retinene, the difference of depression of freezing point due to albumin and retinene, however, appeared to be quite insignificant.

This problem of the nature of the combination between albumin and retinene is now being investigated by another method. A U-tube is divided at the middle with a porous membrans. It is filled with the albumin-retinene complex solution in phosphate buffer and the two compartments are kept at a difference of potential to allow a slow current to pass. The contents of the two compartments are periodically withdrawn and analysed for albumin retinene. This work is in progress.

Though the method of separation of plasma proteins by Cohn's method of 'low-temperatures ethanol separation' gives accurate and reproducible results, but the process is extremely laborious and requires about 30 hours' of dialysis at 4° C. Another method was devised on the basis of the work done by Pillemer *et al.* (1945) for the separation of albumin fraction at a single step. Instead of acetate buffer (pH 6.7 to 6.9) used by the original authors, M/15 mixed phosphate buffer was used and it was found that the percentage of albumin obtained by this method was almost the same as obtained by Cohn's method of separation. After dialysis only for 12 hours, the solution was found to be free from methyl alcohol, as revealed by colour test with Schiff's reagent. After dialysis, the pH was gradually adjusted to 7.5 with N/100 caustic potash and was then combined with retinene as described previously.

The retinene-albumin complex, though stable for a number of days at 4° C. and pH 7.5 (phosphate buffer) was found to be unstable at room temperature. Attempts were made to find out suitable conditions for stabilization of this compound at 37.6° C., so that its influence on living tissue slice can be studied in a Warburg apparatus. Preliminary investigations show that at half the concentration and at a pH 7.8 the compound can be kept unchanged for about six hours at 37.8° C.

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6. Inquiry on the biosynthesis of nicotinic acid under Dr. S. Banerjee at the Presidency College, Calcutta.

STUDIES ON THE BIOSYNTHESIS AND SITE OF SYNTHESIS OF NICOTINIC ACID AND ITS DERIVATIVES FROM TRYPTOPHAN ON RATS KEPT ON NICOTINIC ACID-DEFICIENT DIET.

The dietary tryptophan is converted to nicotinic acid in various mammalian species. The site of this transformation has been claimed by various workers to be in the intestine and in the tissues of the animals. Several workers have shown that when the liver is incubated with tryptophan the later is converted to nicotinic acid. Other observers, however, could not confirm the above finding. In order to obtain information if liver is the site of synthesis of nicotinic acid from tryptophan, the nicotinic acid and some of its derivatives were estimated in the urine of rats kept on : (1) nicotinic-acid-deficient diet followed by (2) nicotinic-acid-deficient diet with extra tryptophan added (one per cent), and (3) the same diet as (2) but the animals being injected intraperitoneally with carbon tetrachloride (0.03 c.c./100 g. body-weight). The latter procedure damages the liver of the animals. Results are given in Table I :—

TABLE I.

Group number	Nature of diet	URINARY EXCRETION (AVERAGE OF SIX DAYS)		
		Nicotinic acid, τ	Quinolinic acid, τ	N ¹ -methylnicotinamide, τ
1	Diet No. 1	9	188	10
	„ 2	40	301	430
	„ 3	24	73	163
2	„ 1	25	258	16
	„ 2	44	516	318
	„ 3	31	102	122
3	„ 1	26	205	16
	„ 2	44	543	739
	„ 3	28	117	212
4	„ 1	16	166	15
	„ 2	52	386	942
	„ 3	38	153	253

From Table I it will be seen that the nicotinic acid, quinolinic acid and N-MN excretions were greatly increased on the addition of tryptophan

in the nicotinic acid-deficient diet. The injection of carbon tetrachloride which damages the liver, completely inhibited the synthesis of quinolinic acid from tryptophan and the synthesis of nicotinic acid and N-MN were also greatly inhibited. It will be seen from the data that the quinolinic-acid and N-MN excretions vary widely from rat to rat although the diet consumption was the same. It might possibly be either due to the difference in the efficiency of the methylating mechanism in the liver or difference in the possible oxidation of N-MN to 6-pyridone. It seems, therefore, that the liver is the principal site for the synthesis of nicotinic acid from tryptophan and the quinolinic-acid excretion may be taken as an index of liver function test.

- (a) *Nicotinic-acid and quinolinic-acid contents of the liver and intestine of normal and carbon tetrachloride-injected rats kept on nicotinic-acid-deficient diet with extra tryptophan added (one per cent) and (b) nicotinic-acid and quinolinic-acid contents of the livers of the same rats incubated with dl-tryptophan.*

Experiment were carried out to see whether the intestine and liver concentration of nicotinic acid differ in two groups of rats : (i) receiving tryptophan-supplemented nicotinic-acid-deficient diet only, and (ii) on the same as diet (i) with a daily intraperitoneal injection of carbon tetrachloride (0.03 c.c./100 g. body-weight). Studies were also undertaken to find if the homogenized livers of these two groups of rats could convert tryptophan to nicotinic acid and quinolinic acid when incubated with tryptophan. The results are given in Tables II and III :—

TABLE II.

Nicotinic and quinolinic-acids content of intestine and liver of normal and carbon tetrachloride-injected rats kept on tryptophan diet for six days.

(The figures are expressed in mg./100 g.).

INTESTINE :				LIVER :			
Normal		Injected		Normal		Injected	
Nicotinic acid	Quinolinic acid	Nicotinic acid	Quinolinic acid	Nicotinic acid	Quinolinic acid	Nicotinic acid	Quinolinic acid
6.0	16.8	6.0	13.9	20.4	65	21.4	56.0
3.5	3.5	3.4	3.83	12.0	53	14.9	24.0
7.1	0	6.2	11.6	19.3	65	11.3	42.0
7.0	7.5	4.2	0	12.9	72	8.7	38.0
4.7	0	6.6	0	11.6	75	12.9	31.0
4.9	11.6	6.9	0	17.4	25	11.5	7.0
5.2	0	6.7	11.3	11.8	41	10.9	23.0
Average:							
5.5	5.6	5.7	5.8	15.05	56.4	13.2	31.4

TABLE III.

Nicotinic-acid content of liver incubated with dl-tryptophan of normal and carbon-tetrachloride-injected rats kept on diet containing dl-tryptophan (one per cent).

(The figures are expressed in mg./100 g.).

NORMAL ANIMALS			CCl ₄ -INJECTED ANIMALS		
Substrate added 10 mg. dl-tryptophan. Incubated for 30 minutes at 37°C.	No substrate added. Incubated for 30 minutes at 37°C.	Substrate added 10 mg. dl-tryptophan. Incubated for 0 minutes.	Substrate added 10 mg. dl-tryptophan. Incubated for 30 minutes at 37°C.	No substrate added. Incubated for 30 minutes at 37°C.	Substrate added 10 mg. tryptophan. Incubated for 0 minutes.
11.8	10.4	11.7	16.0	15.0	15.0
10.0	8.7	9.0	15.7	15.6	16.0
20.0	19.0	21.0	10.5	11.9	11.1
12.6	12.9	12.3	8.0	9.4	9.7
20.2	17.5	20.5	12.6	11.5	11
12.9	13.6	11.5	10.9	9.9	10.6
			10.0	10.8	12.2

Quinolinic-acid content of liver incubated with dl-tryptophan of normal and carbon-tetrachloride-injected rats kept on diet containing dl-tryptophan (one per cent).

(The figures are expressed as mg./100 g.).

NORMAL ANIMALS.			CCl ₄ INJECTED ANIMALS		
Substrate added 10 mg. dl-tryptophan. Incubated for 30 minutes at 37°C.	No substrate added. Incubated for 30 minutes at 37°C.	Substrate added 10 mg. dl-tryptophan. Incubated for 0 minutes.	Substrate added 10 mg. dl-tryptophan. Incubated for 30 minutes at 37°C.	No substrate added. Incubated for 30 minutes at 37°C.	Substrate added 10 mg. tryptophan. Incubated for 0 minutes.
120	66	65	116	...	81
75	51	53	67	51	50
79	67	65	62	49.8	...
127	72	61	11.6	11.6	11.6
102	70	70	41	32	31
74	69	41	38	22	22
27	18.5	15.6	21	18	15

From the results in Table II it will be seen that the nicotinic-acid and quinolinic-acid contents of the intestines of both the groups of rats did not vary at all. Some of the rats of both the groups did not have any quinolinic acid in the intestines. The nicotinic-acid contents of livers of both the groups were also similar, whereas the quinolinic-acid content of the liver of uninjected rats was definitely higher than the rats injected with carbon tetrachloride. Incubation studies showed that normal liver could convert tryptophan more efficiently than the livers of animals receiving injection of carbon tetrachloride. It also appeared that the homogenized livers of both the normal and injected rats could not synthesize nicotinic acid from tryptophan. From these observations it might be said that the quinolinic acid might not be the precursor of nicotinic acid as suggested by some workers.

Metabolism of nicotinic acid and tryptophan in monkeys.—The metabolism of nicotinic acid is not identical in different mammalian species. It has been shown previously that the excretion of N-MN after feeding nicotinic acid is not similar in different species of animals. It is possibly due to the difference in acid-base regulation and clearance mechanism of the kidney or difference in the possible oxidation of N-MN to 6-pyridone. It has also been shown last year that the administration of nicotinic acid does not increase the excretion of trigonelline and 6-pyridone is the major end-product of nicotinic-acid metabolism in rabbits. This year metabolism of nicotinic acid and tryptophan has been studied in monkeys. The results are given in Tables IV, V, VI and VII.

TABLE IV.

Twenty-four hour's urinary excretion of nicotinic acid and its derivatives by monkeys fed germinated gram.

(Figures indicate the percentage of ingested nicotinic acid and its derivatives, excreted in the urine average of three days).

Monkey number	Nicotinic acid	Nicotinuric acid	Quinolinic acid	Trigonelline	N.MN.	6-pyridone
1	5.3	12.1	28.6	14.5	29.7	8.2
2	7.8	12.2	56.6	23.0	52.3	6.8
3	5.6	11.2	54.3	23.0	29.8	6.2

TABLE V.

Twenty-four hours' urinary excretion of nicotinic acid, and its derivatives by monkeys fed with a daily dose of 100 mg. nicotinamide.
(Average of three days).

Monkey number	Nicotinic acid	Nicotinuric acid, mg.	Quinolinic acid, mg.	Trigonelline, mg.	N-MN, mg.	6-pyridone, mg.
1	34.0	1.46	7.36	-0.76	0.850	25.1
2	3.19	1.88	4.25	-0.73	0.375	52.5
3	4.93	5.01	7.20	-1.30	0.347	32.5

TABLE VI.

Twenty-four hours' urinary excretion of nicotinic acid and its derivatives by monkeys by feeding a daily dose of 100 mg. dl-tryptophan.

(Average of five days).

Monkey number	Nicotinic acid, mg.	Nicotinuric acid, mg.	Quinolinic acid, mg.	Trigonelline, mg.	N-methyl-nicotinamide, mg.
1	0.18	-0.02	1.45	0.56	47.4
2	0.12	0.09	0.93	0.61	57.8
3	0.13	0.09	0.83	1.30	92.6

TABLE VII.

Twenty-four hours' urinary excretion of nicotinic acid and its derivatives by monkeys by feeding a daily dose of 100 mg. dl-tryptophan and 1 gm. sulphaguanidic.

(Average of six days).

Monkey number	Nicotinic acid, mg.	Nicotinuric acid, mg.	Quinolinic acid, mg.	Trigonelline, mg.	N-meth-nicotinamide, mg.
1	0.03	0.066	-1.10	-0.54	-8.6
2	Trace	0.03	-1.64	-0.52	-11.5
3	0.06	0.06	-1.99	-0.26	-16.5

It will be seen from the tables that monkeys excreted 14 to 23 per cent of its dietary trigonelline in their urine and the trigonelline excretion remained practically the same after administration of nicotinamide and tryptophan. It will also be seen that the tryptophan at the level of 100-mg. dose did not appreciably increase the quinolinic-acid and nicotinic-acid excretion and the administration of sulphaguanidine decreased the excretions of all the metabolites of nicotinic acid and 6-pyridone was found to be the major end-product of nicotinic-acid metabolism in monkeys.

Isolation of combined nicotinic acid from fish-muscle tissue.—It has been reported previously that the nicotinic-acid value of the fish tissues, when hydrolysed with normal alkali, was higher when the same is hydrolysed with normal acid. It has also been shown that the increased value is not due to the release of nicotinic acid from D.P.N. which is equally hydrolysed with normal acid and alkali. From these observations it was considered that the fish tissues contain one bound form of nicotinic acid similar to that present in the cereals. This year attempts have been made to isolate the substance by using various solvents and adsorbents and precipitating with ammonium sulphate and phosphotungstic acid. The results are given in Table VIII :

TABLE VIII.

Nicotinic-acid content of the dried water extracts of the fish-muscle tissues after extraction with different solvents, adsorbents and precipitants.

(Figures are expressed in mg./100 g. of the dried extract).

	Initial	After extraction	Residues
A. Alcohol—			
N.HCl hydrolysis ...	3.42	2.6	0.53
N.NaOH hydrolysis ...	5.05	4.2	0.90
Acetone			
N.HCl hydrolysis ...	1.57	1.32	0.21
N.NaOH hydrolysis ...	2.53	1.93	0.20
Methyl alcohol			
N.HCl hydrolysis ...	1.57	1.37	0.12
N.NaOH hydrolysis ...	2.53	2.24	0.20
Chloroform			
N.HCl hydrolysis ...	2.19	0.17	1.90
N.NaOH hydrolysis ...	3.51	0.34	2.87
Ether			
N.HCl hydrolysis ...	2.19	0.40	2.06
N.NaOH hydrolysis ...	3.51	0.57	2.81
Benzene			
N.HCl hydrolysis ...	2.19	traces	2.06
N.NaOH hydrolysis ...	3.51	traces	3.24
Carbontetrachloride			
N.HCl hydrolysis ...	2.19	traces	2.05
N.NaOH hydrolysis ...	3.51	traces	3.42
Ether and alcohol (1 : 1)			
N.HCl hydrolysis ...	2.19	2.2	Nil
N.NaOH hydrolysis ...	3.51	3.44	Nil
B. Treatment with different adsorbents			
Active carbon (one per cent)			
		Filtrate fraction	
N.HCl hydrolysis ...	2.6	0.22	
N.NaOH hydrolysis ...	3.90	0.24	
Activated alumina			
N.HCl hydrolysis ...	2.6	1.9	
N.NaOH hydrolysis ...	3.9	3.4	
Kieselguhr (one per cent)			
N.HCl hydrolysis ...	5.6	4.99	
N.NaOH hydrolysis ...	8.06	7.80	
C. Treatment of ammonium sulphate and phosphotungstic acid.			
Ammonium sulphate (50 per cent)			
		Filtrate	
N.HCl hydrolysis ...	2.6	2.4	
N.NaOH hydrolysis ...	3.9	3.92	
Phosphotungstic acid (20 per cent)			
N.HCl hydrolysis ...	3.12	0.12	
N.NaOH hydrolysis ...	5.30	0.50	

It will be seen from Table VIII that the bound form of nicotinic acid present in fish-muscle tissues is soluble in alcohol, acetone and methyl alcohol and insoluble in chloroform, ether and benzene and carbon tetrachloride. Active carbon adsorbs both nicotinic acid and the bound substance, whereas activated alumina and Kieselguhr slightly adsorbed the free nicotinic acid and the bound one. It will also be found that phosphotungstic acid completely absorbed both the forms of nicotinic acid and combined nicotinic acid was not precipitated with ammonium sulphate.

7. Inquiry on the enzymic rôle of inositol under Dr. P. S. Sarma at the University Biochemical Laboratory, University of Madras, Madras.

The object of this investigation is to find out if inositol has an essential rôle to play in the activity of alpha amylase. It was reported last year that (1) the inhibition of the synthesis and secretion of amylase produced by the gamma isomer of hexachlorocyclohexane is reversed not only by inositol but by biotin as well, and (2) in *ueurospora crassa*, the inhibition of growth produced by the gamma isomer is reversed by biotin in the wild strain, whereas in the mutant strain, the reversal by inositol is more complete in the presence of biotin than in its absence. These results suggest a close relationship between inositol and biotin and this has been investigated further. It was thought that biotin may act in this manner by virtue of a direct rôle in the activity of alpha amylase or by stimulating in some manner the synthesis and utilization of inositol.

In order to determine if biotin has a direct rôle in the activity of alpha amylase, biotin deficiency was produced in rats and rice-moth larvæ by feeding a diet containing egg white and the variation in amylase activity was noted. The results obtained are given in Tables I and II. In series I

TABLE I.

AMYLASE ACTIVITY (UNITS OF SMITH & ROE)			
	Egg-white diet	Egg white + biotin	Egg white + inositol
(a) In blood per 100 c.c. Series I	Number of rats : 4 Range: 2193-2427 Average : 2327	Number of rats : 4 Range: 2113-2711 Average : 2341	Number of rats : 4 Range: 1968-2264 Average : 2160
Series II	Number of rats: 6 Range: 3326-3541 Average : 3467	Number of rats: 5 Range: 2711-3722 Average : 3324	Number of rats: 4 Range: 3127-3953 Average : 3528
(b) In pancreas (per mg. acetone-dry pancreas) Series I	Number of rats: 4 Range: 20.6-38.0 Average : 30.1	Number of rats: 4 Range: 27.8-32.8 Average : 30.0	Number of rats: 4 Range: 78.8-92.4 Average: 84.2
Series II	Number of rats: 6 Range: 27.4-36.0 Average : 30.5	Number of rats: 5 Range: 24.8-37.6 Average : 30.3	Number of rats: 4 Range: 25.8-31.6 Average : 28.4

the composition of the diet was as follows : Rice powder, 94 g. ; salt mixture, 6 g. ; vitamin A, 200 I.U. ; vitamin E, 2.24 mg. ; thiamin, 0.3 mg. ; riboflavin, 0.5 mg. ; nicotinic acid, 0.4 mg. ; calcium pantothenate, 2.0 mg. ; choline chloride, 150 mg./100 g. diet ; egg white was added at a level of 150 c.c. /100 g. diet, While in series II, the diet used was that described by Nielsen & Elvehjem (*Jour. Biol. Chem.*, **144**, p. 405, 1942), with egg white being added at the same level as in the other diet. Biotin was given to the control rats at the rate of 10 τ /rat/day and 100 τ /week intra-peritoneally. Inositol was added at a level of 1 g./100 g. diet.

TABLE II.

Amylase activity of biotin-deficient and biotin-fed larvæ.

Diet	Number of larvæ/dish.	Weight in mg./10 larvæ	AMYLASE ACTIVITY/MG. ACETONE-DRY LARVÆ TISSUE :	
			Smith & Roe units	Somogyi units
(a) Biotin-deficient larvæ (Egg-white diet)	40	53.4	0.985	1.8
	40	55.0	0.935	1.9
(b) Biotin-fed larvæ (biotin 100 τ /1 week)	40	145.2	1.10	2.0
	40	160.2	1.06	1.92
(c) Whole-wheat diet ...	40	190.2	1.07	2.13

It will be seen from the results given above that there are no significant differences between the amylase activity of biotin-deficient and biotin-fed rats, on the one hand, and between biotin-deficient and biotin-fed larvæ on the other. It appears, therefore, that biotin has no direct rôle in the activity of alpha amylase.

It will, however, be observed that in series I there is a marked increase in pancreatic-amylase activity in rats fed egg white and inositol. Further, these rats showed severe signs of alopecia. This marked accumulation of amylase in the pancreas appears to be of interest and has been investigated further.

Studies with pigeon-pancreas slices were carried out to see if any increase in the synthesis and secretion of amylase took place in the presence of egg white or the antibiotin compound (τ -(3, 4-Ureylene cyclohexyl)-butyric acid and inositol. The method of preparing the slices and incubating them was the same as described in the previous report. In some experiments ATP was also added to the incubation medium to see if it has any effect. The results obtained are given in Tables III and IV:—

TABLE III.

Incubation time : 2 hours ; in 2 c.c. sheep serum.

Supplements	AMYLASE ACTIVITY (SMITH & ROE UNITS) : MG. DRY TISSUE		
	Tissue	Medium	Total
1. Control ...	69.0	51.8	120.8
2. 1 c.c. egg white ...	85.1	59.3	144.4
3. 1 c.c. egg white + 100 mg. inositol ...	102.3	94.0	196.3
4. 2 c.c. egg white ...	69.1	71.6	140.7
5. 2 c.c. egg white + 200 mg. inositol	63.7	79.7	143.4

TABLE IV.

Incubation period : 3 hours ; in 2 c.c. sheep serum.

Supplements	AMYLASE ACTIVITY (SMITH & ROE UNITS/ MG. DRY TISSUE)		
	Tissue	Medium	Total
1. Control + 3 mg. ATP ...	118.6	39.7	158.3
2. 3 mg. antibiotin + 3 mg. ATP	108.7	59.6	167.3
3. 3 mg. antibiotin + 3 mg. ATP + 200 mg. inositol ...	118.9	74.7	193.6
4. 3 mg. antibiotin + 3 mg. ATP + 200 mg. inositol	113.6	64.9	178.5
5. 1 c.c. egg-white + 3 mg. ATP + 200 mg. inositol ...	125.5	102.3	227.8

These results have been repeated several times to test the reliability of the results obtained. Though the results obtained, so far, have not been entirely consistent from one set of experiments to another, it will be seen from the results given above that there is a greater increase in the amylase synthesised in the presence of egg white or the antibiotin compound, and inositol. More consistent results have, however, been obtained in the presence of adenosine triphosphate, the exact rôle of which in this respect has yet to be determined.

In view of the results already obtained with inositol-deficient rats, inositol deficiency was produced in rice-moth larvæ by the addition of the gamma isomer of hexachlorocyclohexane to the diet. The gamma isomer was dissolved in benzene and added at a level of 2 mg. per 100 g. diet,

The basal diet used had the same composition as described by Sarma (*Ind. Jour. Med. Res.*, 1943, **31**, p. 165). Amylase activity of acetone-dry larvæ tissue was estimated by the method of Somogyi (*Jour. Biol. Chem.*, 1938, **125**, p. 399) and Smith & Roe (*Jour. Biol. Chem.*, 1949, **179**, p. 53).

TABLE V.

Diet	Number of larvæ per dish	Weight of larvæ in mg.	Amylase activity	
			Smith & Roe units	Somogyi units
1. Inositol-defecient larvæ ...	30	95·8	1·56	3·91
(2 mg./100 g. diet) ...	30	93·4	1·61	3·52
2. Inositol-containing diet ...	30	204·2	2·07	5·5
(2 mg./100 g. diet) ...	30	195·2	2·13	5·3
+100 mg./100 g. diet)				
3. Whole-wheat diet ...	30	265·8	2·42	5·04
	30	271·6	2·21	4·93

It will be seen from the results in Table V that inositol defecency in rice-moth larvæ is accompanied by a lowering of the amylase activity and this inhibitory effect is reversed by inositol.

Further work has been done with rabbits to test if the inhibition of amylase activity of blood produced by the gamma isomer of hexachlorohexane is reversed by inositol more completely when given as intraperitoneal injections than when fed orally. The results given in Table VI show that inositol is just as effective, if given by injection, as by oral feeding and that biotin has no effect in reversing the inhibition by gamma isomer.

TABLE VI.

AMYLASE ACTIVITY/100 C.C. BLOOD (SOMOGYI UNITS)

Control	Gammexane (250 mg.) fed	Gammexane + Inositol (ig) (Oral)	Gammexane + inositol (Injection)	Gammexane biotin (200 τ) (Oral)
190	145	182	180	148
185	137	187	177	138
188	142	177	172	142
194	134	139

Experiments were carried out to see if inositol by itself is able to enhance the amylase activity of rabbits blood. The results given in Table VII show that feeding of inositol alone has no effect on amylase activity,

probably due to the fact that the animal has enough inositol in its body for the purpose.

TABLE VII.

AMYLASE ACTIVITY (SOMOGYI UNITS/100 c.c.)	
Control	Inositol fed (2,500 mg.)
198	188
204	195
195	198
193	192

8. Inquiry on generalized mechanism of action of vitamin A in the body under Dr. D. P. Sadhu at the Presidency College, Calcutta.

(1) *Study of tissue respiration in vitamin-A deficient and hypervitaminosis A rats.*—Last year it was reported that vitamin A influences the tissue-redox system by affecting the distribution of ascorbic acid and glutathione in the body. The redox system influences the distribution of cystine: cysteine ratio to form sulphuric acid and oxidation and amination of sugars to form hexuronic acid and hexosamines. This year investigations were undertaken to see whether vitamin A has any influence on the tissue respiration.

Thirty weanling rats were placed on vitamin-A deficient diet and divided into three groups. One group received vitamin A in the form of water-soluble vitamin A (Roché) once a week and grew normally, hereafter called the control group. One group received 10,000 I.U. vitamin A for four weeks and the other group took deficient diet till the deficiency signs, such as typical eye lesions, developed. The animals were then sacrificed by decapitation. Succinic de-hydrogenase activity and lactic de-hydrogenase activity in the liver, kidney and intestine were studied by the Thunberg technique. The results are given in Table I :—

TABLE I.

Effect of vitamin-A feeding on tissue respiration of three groups of rats.

(Figures indicate time in seconds taken to cause a 90 per cent reduction in the colour of methylene blue per gramme of tissue).

Average of ten animals in each group.

		Succinic de-hydrogenase			Lactic de-hydrogenase		
		N	D	H	N	G	H
Liver	13·5	10·6	14·0	21·8	16·2	38·6
Kidney	...	11·5	10·5	14·6	10·5	9·7	11·9
Intestine	...	23·1	20·3	33·7	24·4	21·9	27·3

The results in Table I show that there is a tendency for these enzymic activities to be increased in hypovitaminosis-A and decreased in hypervitaminosis-A rats. This indicates that tissue respiration is affected by vitamin A.

(2) *Sulphur partitioning of the urine in normal, hypovitaminotic and hypervitaminotic rats.*—Three groups of rats were maintained in the similar way as normal, vitamin-A deficient and hypervitaminotic. Urines were regularly collected from all the groups with dilute hydrochloric acid and toluene, and analysed for the different forms of sulphur, viz. inorganic sulphur, etherial sulphur and neutral sulphur. The sulphur partitioning was studied during the progressive stages of the development of vitamin-A deficiency and hypervitaminosis A. The results are given in Table II :—

TABLE II.

Daily excretion of different forms of sulphur in urine expressed as sulphate ions in mg. per rat.

CONTROL:			HYPERVITAMINOTIC.			DEFICIENT.		
Inorg-anic.	Ethe-real.	Neut-ral.	Inorg-anic.	Ethe-real.	Neut-ral.	Inorg-anic.	Ethe-real.	Neut-ral.
2·11	0·09	0·03	2·08	0·09	0·02	2·14	0·12	0·04
2·25	0·12	0·02	2·15	0·12	0·04	1·44	0·19	0·07
2·17	0·26	0·04	3·04	0·11	0·03	2·11	0·32	0·08
2·25	0·19	0·06	3·12	0·15	0·07	2·05	0·42	0·07
2·38	0·17	0·05	3·15	0·16	0·08	1·95	0·56	0·09
2·56	0·21	0·04	3·00	0·18	0·05	1·80	0·62	0·10
2·65	0·21	0·08	2·85	0·19	0·09	1·62	0·60	0·11
2·81	0·24	0·08	2·75	0·18	0·08	1·49	0·58	0·12
3·07	0·22	0·07	2·70	0·19	0·09	1·36	0·71	0·13
3·12	0·19	0·05	2·65	0·16	0·09	1·05	0·75	0·10
3·08	0·24	0·08	2·52	0·15	0·08	1·14	0·82	0·14
3·12	0·24	0·09	2·08	0·18	0·07	1·08	0·81	0·15

It will be noticed from the results that the inorganic sulphur excretion in all the groups varied. It was, however, due to the changes in the food intake. Inorganic sulphur per gramme of food intake did not, however, significantly vary in any one of the groups. In the group developing vitamin-A deficiency, the etherial sulphur fraction, however, increased progressively and the excretion of the neutral sulphur fraction also was higher in this group in comparison to the other two groups. In the group developing hypervitaminosis A, the etherial and neutral sulphur fractions were not significantly different from the normal group, though a tendency to excrete less etherial sulphur is noticed in this group.

(3) *Sulphur content of different tissues in normal hypo-A and hyper-A rats.*—After the animals developed acute vitamin-A deficiency and hypervitaminosis A, they were sacrificed along with the normal rats and sulphur content of the liver, skin and the remaining parts of the body were determined to find out the storage of sulphur in the body of the three grouped of rats. Total

body-sulphur was estimated by digesting the carcasses with Pirie's reagent. The results are given in Table III :—

TABLE III.

Total sulphur content of tissues in three groups of rats.

(Figures indicate mg. of sulphur per 100 g. of tissue. Average of six animals in each group).

Treatment	Liver	Skin	Total body
Control ...	200 \pm 9.8	350 \pm 13.2	190 \pm 11.3
Hypervitaminosis ...	250 \pm 12.5	368 \pm 13.2	210 \pm 10.5
Vitamin A deficiency ...	185 \pm 10.2	300 \pm 12.5	180 \pm 9.8

The results in Table III will show that vitamin A influences the storage of sulphur in the tissues. In vitamin-A deficiency, there is decreased content of total sulphur in liver, skin and the total body, whereas in hypervitaminosis A, there is increased content of sulphur in the different tissues.

(4) *Urinary sulphur partitioning and body-sulphur estimation of normal hypo-A and hyper-A rats maintained on diets free from inorganic sulphur.*—The whole series of experiment were repeated with similar three groups of rats but maintained on diets free from inorganic sulphur, which was present in the earlier experiment along with the salt mixture. Sulphate compounds were eliminated from the salt mixture and replaced by other sulphur-free compounds and the urines regularly collected and analyzed for different forms of sulphur. The results were given in Table IV :—

TABLE IV.

Daily excretion of different forms of sulphur in urine (expressed as sulphur ions in mg. per rat) by three groups of rats maintained on inorganic sulphur-free diet.

CONTROL			HYPERVITAMINOTIC			DEFICIENT		
Inorganic	Ether- ial	Neut- ral	Inorg- anic	Ethereal	Neutral	Inorga- nic	Ether- eal	Neut- ral
0.18	0.12	0.04	0.25	0.10	0.04	0.19	0.11	0.04
0.22	0.14	0.02	0.28	0.09	0.02	0.18	0.13	0.05
0.25	0.16	0.03	0.24	0.11	0.04	0.24	0.18	0.06
0.24	0.17	0.05	0.25	0.13	0.05	0.25	0.22	0.04
0.28	0.21	0.06	0.29	0.16	0.07	0.28	0.30	0.08
0.31	0.20	0.08	0.32	0.18	0.08	0.22	0.35	0.07
0.29	0.19	0.07	0.34	0.17	0.05	0.18	0.41	0.09
0.32	0.20	0.05	0.35	0.15	0.04	0.15	0.48	0.11
0.33	0.22	0.06	0.37	0.14	0.05	0.13	0.55	0.08
0.34	0.23	0.08	0.33	0.16	0.08	0.11	0.58	0.09

It will be noticed from the results that only the inorganic sulphur fraction has been greatly reduced, while the excretions of the other two fractions were similar as in the earlier experiment. The increased excretion of ethereal sulphur fraction in the vitamin-A deficient animals was again observed and confirmed.

9. Inquiry on the determination of sodium and potassium of common Indian foodstuffs and the preparation of low-sodium high-potassium diets for different clinical conditions under Prof. K. Rajagopal and Dr.

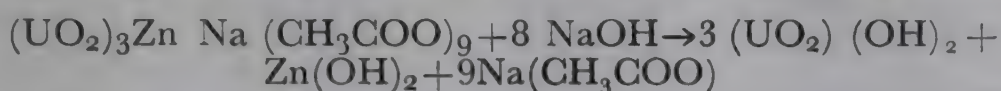
(Smt.) B. Banerjee at the All-India Institute of Hygiene & Public Health and Medical College Hospitals, Calcutta.

(1) Analysis of foodstuffs for the sodium and potassium values were carried out in seventy-three samples procured from the local markets. From among these workable diet schedule can be formulated.

The methods used for analysis were :—

(i) *For sodium*.—Method of Weinbach—precipitation of sodium as sodium zinc-uranyl acetate and titration with sodium hydroxide.

The sodium is precipitated as triple salt-uranyl-zinc-sodium acetate and subsequently the salt is titrated with sodium hydroxide as follows :—



(*Jour. Biol. Chem.*, 1935, **110**, p. 95)

(ii) *For potassium*.—Platinic-chloride method is followed.

This method is applicable in the presence of chlorides of sodium, lithium magnesium, cobalt and strontium.

Principle.—Potassium chloroplatinate is practically insoluble in strong alcohol, while the other chloraplatinates are readily soluble.

(*A.O.A.C.*, p. 98).

Analysis of foodstuffs.—Seventy-three foodstuffs in the raw state have been analysed for sodium and potassium values. The range of these values is given below.—

(ii) The figures determined can be conveniently grouped into their average sodium values ranging from 0 to 5 mg./100 g., 6 to 10 mg./100 g., 11 to 20 mg./100 g., 21 to 50 mg./100 g., and 51 to 100 mg./100 g.

TABLE I.

Showing foodstuffs arranged in different range of sodium content.

Group I Foodstuffs containing sodium from 0-5 mg./100 g.	Group II Foodstuffs containing sodium from 6 mg. to 10 mg./100 g.	Group III Foodstuffs containing sodium from 11 mg. to 20 mg./100 g.	Group IV Foodstuffs containing sodium from 21 mg. to 50 mg./100 g.	Group V Foodstuffs containing sodium from 51 mg. to 100 mg./100 g.
<i>Fruits :</i> Lime juice	<i>Vegetables :</i> Drumstick	<i>Fruits :</i> Water chest nut	<i>Vegetables :</i> Yam	<i>Vegetables :</i> Lady's finger
Custard apple	Calabash	Dates	Colacasia	Spinach
Plantain	Cucumber	Guava	Radish	
Apples	Lettuce		Carrot	
Grapes	Beans (French)			
Cucumber				
Pomegranate				
Sweet lime				
Papaya (ripe)				

TABLE I—(contd.)

Group I Foodstuffs containing sodium from 0-5 mg./100 g.	Group II Foodstuffs containinig sodium from 6 mg. to 10 mg. /100 g.	Group III Foodstuffs containing sodium from 11 mg. to 20 mg./ 100 g.	Group IV Foodstuffs containing sodium from 21 mg. to 50 mg./100g.	Group V Foodstuffs containing sodium from 51 mg. to 100 mg/100g
<i>Vegetables</i> Ash gourd Pumpkin	<i>Cereals</i> Rice fine quality Rice home pounded Cholum Bajra	<i>Vegetables</i> Knol khol Bitter gourd	<i>Dry fruits</i> Cashew nuts Prunes	<i>Dry fruits</i> Raisins Apricots
Amla Green- plantain Patol Tomatoes Coriander leaves (green) Onion		Potatoes		
Vegetable marrow CEREALS Coarse rice Polished rice Barley		<i>Cereals</i> Wheat White flour (maida)	<i>Pulses</i> Horse gram Lentil <i>Dairy products:</i> Milk Skimmed milk	<i>Dairy products:</i> Channa Egg yolk Egg whole Egg white FLESHY FOODS Meat Fish <i>Dry pulses:</i> Green gram Cow gram Field beans Black gram Bengal gram Red gram

Processing.

The following foodstuffs were cooked by boiling in water and the loss of sodium and potassium during the process are summarized below :—

- (i) Potatoes
- (ii) Rice
- (iii) Cereals
- (iv) Pulses

I. *Potatoes*.—The potatoes were boiled in just sufficient water for 15 to 20 minutes and cooked in the following states :—

- (i) Peeled.
- (ii) Peeled and cut into average small pieces.
- (iii) With skin.

On boiling the potatoes there was increase in weight when they were cut into pieces. On the contrary, there was little loss in weight about four per cent when boiled as a single large whole potatoes.

TABLE II.

Showing loss of sodium and potassium in potatoes during processing by boiling method.

Number	Name of the food article	Per 100 g. as raw		Loss per cent	
		Na in mg.	K in mg.	in Na	in K
1.	Raw potatoes ...	17	257
2.	Boiled peeled potatoes ...	15.6	201	8.23	21.80
3.	Boiled peeled and cut into average small pieces ...	11.3	146	33.53	43.20
4.	Boiled potatoes with skin ...	16.4	178	3.50	30.74

Effect of time on boiling of potatoes varying from $\frac{1}{2}$ hour to one hour showed that there was more loss of sodium when boiled for a longer period.

II. *Rice*.—Rice boiled in large quantity of water and when the rice was cooked and the water discarded, the loss in sodium amounted to 14.70 per cent.

III. *Cereals*.—A method for rendering cereals, other than rice, low in sodium was studied. The three different mixtures of flours of Bajra, cholum and barley incorporated with wheat flour, in different proportions were studied, as follows :—

			Mixture I	Mixture II	Mixture III
			Part	Part	Part
Bajra	1	1	1
Wheat	1	1	1
Cholum	2	2	1
Barley	2	...	3

The wheat flour was added to enable the dough to knead easily. The dough was placed in boiling water for five to eight minutes. After removing it the values for sodium and potassium were studied. There was loss in both the minerals.

	Mixture I, per cent	Mixture II, per cent	Mixture III, per cent
Loss in sodium ...	17.83	15.40	16.0
Loss in potassium ...	25.34	20.10	23.51

The doughs of these mixtures were similarly studied for sodium and potassium values, by boiling in water and adding a known amount of K

salt. In this case there was more loss of sodium and at the same time a relative increase in K. The loss of sodium varied from 30 to 38 per cent the increase in potassium varied from 13 to 20 per cent.

After processing the cereals in this way, chapattis (as made in common Indian way) were made. The cooking properties and taste of the preparation were good.

Mixture III was finally chosen and studied in duplicate. The average results are represented below :—

	Per cent
(i) Loss in sodium from the foodstuff without the addition of K salt	16
(ii) Loss of potassium from the foodstuff without the addition of K salt	23.51
(iii) Loss of sodium from the foodstuff (0.20 g. KCl for 25 g. of flour) when boiled with K salt added in water ...	38.43 -
(iv) Gain in potassium in the foodstuff	20.52

IV. *Pulses*.—Four pulses, viz. black gram, Bengal gram, green gram red gram, were first analysed as raw diets, i.e. (i) with husk and (ii) without and husk. A difference for increase in potassium content by 10 to 23 per cent was noted.

Processing of dhals.—The dhals were first boiled in water without the addition of K salt. The water was allowed to boil for eight to ten minutes before the pulses were added. Pulses were allowed to boil from 10 to 15 minutes as the pulses just soft and semi cooked. The water was strained through a sieve and analysed. During this process there was a loss of sodium from 24 to 31 per cent in the foodstuff.

When dhals are cooked in a similar way but with the addition of KC in boiling water there was more loss in sodium and gain in potassium contents.

The results were :—

	Per cent
(1) Loss in sodium in the foodstuff by boiling without the addition of K salt	23.73 to 30.81
(2) Loss in potassium in the foodstuff by boiling without the addition of K salt	16.84 to 22.55
(3) Loss of sodium in the foodstuff boiling in water and with the addition of KCl ...	29.60 to 38.12
(4) Gain in potassium in the foodstuff when K salt is added in boiling water	15.10 to 62.43

There was more loss of sodium in the foodstuff when boiled in water with the addition of K salt. It varied from six to eight per cent more than the loss of sodium when only boiled in water.

- (i) Amount of KCl used 0.300 g. or 157 mg. of potassium.
- (ii) Weight of pulse taken for processing 10 g.

10. Inquiry on human requirements of riboflavin and thiamin under Dr. H. N. De at the M. G. M. Medical College, Indore.

During the period under review, the work on the assessment of riboflavin requirements of the normal Indian adults and other aspects of the requirement values was continued on the following lines :—

STUDIES BASED ON TYPICAL INDIAN DIETARIES

After determining the riboflavin content of the chosen typical Indian dietaries, such as vegetarian with rice, wheat and jowar diets and non-vegetarian with rice and wheat diets as consumed by the middle-class Indians of different dietary habitat, the adequacy of these diets as to the fulfilment of riboflavin requirements of normal Indian adults has been thoroughly studied by employing a large number of experimental subjects. The vegetarian diets contained 450 g. of cereals (wheat, rice or jowar), 100 g. of pulse, 220 g. of vegetables (rooty and leafy) and 30 g. of oil. In case of non-vegetarian diet the pulse was substituted by equal amount of mutton (muscles).

The technique of determining the riboflavin requirement was the same as reported last year. The subjects were given the same diet during the whole of the experimental period which lasted for more than two months. Employing the previously described microbiological method for estimation of riboflavin, the 24-hour urinary output of riboflavin was determined after—

- (1) One week has passed on the particular chosen diet (basal excretion period—P-I).
- (2) Administering a single 5-mg. test dose of riboflavin at the end of the week (basal excretion on test-dose period—P-II).
- (3) Giving 10 mg. of riboflavin as daily dose for one week followed by a single 5-mg. test dose (saturation period—P-III).
- (4) Similar treatment for one more week followed by a single 5-mg. test dose (saturation period—P-IV).
- (5) Giving 2 mg. of riboflavin as daily dose for one week followed by a single 5-mg. test dose (experimental period—P-V).
- (6) Giving 1 mg. of riboflavin as daily dose for one week followed by a single 5-mg. test dose (experimental period—P-VI).
- (7) Giving only basal diet without any riboflavin supplement for one week followed by a single 5-mg. test dose (experimental period on basal diet—P-VII).

The percentage excretion of the test dose was calculated each time and this served as an index as to the saturation stage of the body-tissues with regard to riboflavin.

From the results of the saturation experiment as represented in Table I, it seems apparent that the diets mentioned above, which supplied from a minimum of 0.83 mg. to the maximum of 1.21 mg. of riboflavin were fairly adequate to satisfy the saturation requirement of the vitamin for normal adults of body-weights varying from 50 to 57 kilos. Even at a low level of intake of 0.83 mg. as supplied by the vegetarian jowar diet,

the subjects could maintain the saturation level and this suggests that the riboflavin requirement of normal Indian adult may possibly be fulfilled even by low intake of 0.83 mg. Before coming to any definite conclusion about requirement values of Indian adults it is, however, necessary to explore the minimum requirement of riboflavin below which the body will fail to maintain the saturation level. Information in this line may be gathered if a number of experimental subjects are kept on the diets with riboflavin supply varying from 0.4 mg. to 0.6 mg. per day for a prolonged period of six to nine months (to be extended if necessary). At frequent intervals the urinary output of riboflavin is to be measured until it reaches to fairly constant level. Saturation state will then be determined at this stage of low riboflavin intake. If the saturation level is not maintained at this stage, riboflavin intake will have to be increased gradually until the saturation level is reached. Since in such dietary planning there is probability of low intake of protein which might affect the riboflavin excretion and hence requirement values, it is, therefore, necessary to supplement such diet with vitamin-free casein which will balance the protein level without affecting the riboflavin level of the diet.

A diet was formulated in the above way but since tapioca could not be procured in large quantity for this work the experiment was not started. This important work will be taken up as soon as sufficient tapioca (unfortified) is available in hand.

URINARY EXCRETION OF RIBOFLAVIN ON DIFFERENT DIETARIES.

Results presented in Table I show that on the vegetarian (with wheat, rice and jowar) diets the riboflavin excretion varied from 103 μ g. to 181 μ g. per day and the values calculated as percentage of intake ranged from 8.5 to 19.8. On the non-vegetarian diet with rice or wheat and mutton the range of riboflavin excretion was found to be at a higher level of 238 μ g. to 293 μ g. per day and the values calculated as percentage of intake ranged from 24.5 to 27.7.

From the results, it appeared striking that the riboflavin excretion on non-vegetarian diet was higher than that on vegetarian diet. This increase in excretion due to the substitution of pulse by mutton in the diet may be either due to the change in the protein-level of the diet or due to the effect of higher biological value of mutton protein as compared to that of pulse protein. Since in all these experiments protein level of the diet was not measured before and moreover non-vegetarian and vegetarian diets were given to different subjects in different sets of experiment instead of substituting the pulse by mutton in the same experiment, it is, therefore, not possible to generalize anything from the above values. In order to study this point thoroughly the following experiments were performed under restricted dietary conditions :—

EFFECT OF SUBSTITUTION OF PULSE PROTEIN BY ANIMAL PROTEIN ON THE RIBOFLAVIN EXCRETION AND REQUIREMENT.

Three selected subjects were first given a vegetarian rice or wheat diet and after a preliminary period of four days on this diet, the daily urinary excretion of riboflavin was measured for three consecutive days. The pulse (100 g.) was then substituted by an equal amount of mutton (muscles) and after the preliminary period of four days on this diet the daily urinary excretion of riboflavin was determined as before for three consecutive days. Dietary

protein and riboflavin levels were also measured in all cases. It will be observed from the results in Table II that the substitution of pulse by equal amount of mutton does not affect the riboflavin content but decreases the protein content from 63.9 g. to 57.4 g. in the case of rice diet and from 77.4 g. to 66.8 g. in the case of wheat diet. While surveying the riboflavin excretion on these diets it becomes evident that substitution of pulse protein by mutton protein leads to an increase of riboflavin excretion from 9.4 g. to 17.4 g. when calculated in terms of percentage of the dietary intake. This apparently leads one to conclude that the drop in the protein level might have produced this increased excretion but the probability that this change might also be due to higher biological value of mutton protein as compared to that of pulse protein cannot be over ruled. Although sufficient literature, mostly based on animal experimentation has accumulated to show that the level of the protein in diet influences the riboflavin excretion and requirement it is not yet definitely known how far the change in quality of protein in the diet affects the riboflavin excretion and requirement. Since one of the main functions of riboflavin is its participation in de-amination of amino acids (1-also) it is not improbable to envisage that the animal protein for the utilization of which, less de-amination is involved than in the case of pulse protein of lower biological value, may spare the dietary riboflavin producing increased excretion in urine. In order to get correct information in this line a set of experiment has been started in which the pulse was substituted by meat in the proportion keeping the protein and riboflavin contents equal. The riboflavin excretion due to such change in the quality of the protein will help us to gather the correct information as to how far the biological value of the protein affects the riboflavin requirement. The faecal nitrogen and urinary total, urea, and uric acid nitrogen and also total sulphur, inorganic sulphate, etherial sulphate and neutral sulphur will be estimated which might throw new light in this important aspect of riboflavin requirement.

RIBOFLAVIN LEVEL OF BLOOD OF NORMAL INDIAN ADULT.

The condition of the population with regard to their dietary adequacy for satisfying their riboflavin requirement may be assessed not only by saturation test dose experiment of the urinary excretion, but also by the riboflavin content of the blood. Although some data are available for the people of the Western countries, there is no such data for the Indians of different age groups and sex. It is, therefore, felt necessary to study the riboflavin level of blood of the normal individuals, which might help us to set a normal standard values from which it will be possible to assess the ariboflavinosis condition easily in a group of population. Within a short period available at our disposal it was possible to study the values of riboflavin content of blood (fasting blood) of 16 normal adults. The results presented in the Table III show that the range of riboflavin in blood of normal Indian adults of ages from 20 to 33 years varies from 0.48 $\mu\text{g./c.c.}$ to 0.6 $\mu\text{g./c.c.}$ More values are being collected for assessing standard level for normal male and female adults, children (boys and girls), infants and pregnant lactating women. The partition of riboflavin—both free and combined in serum, r.b.c. and w.b.c., will also be investigated side by side. In our future experiments for assessing the minimum requirement the above standard values will guide us to judge the condition of the subject when they will be kept for a prolonged period on low riboflavin diet.

TABLE I.

Name of subject, age and weight.	Nature of diet.	Dietary intake of riboflavin mg.	P—I.		P—II.		P—III.		P—IV.		P—V.		P—VI.		P—VII.	
			Basal excretion of riboflavin in μ g.		Basal excretion on test dose of 5 mg. in μ g.		Saturated period with 10 mg. daily dose, and 5 mg. Test dose.		Experimental Period		Daily dose, 2 mg. and Test dose, 5 mg.		Daily dose, 1 mg. and Test dose, 5 mg.		Basal diet and Test dose, 5 mg.	
			Total	Intake per cent.	Total	Test dose, per cent	Total excretion in μ g.	Test dose, per cent.	Total excretion in μ g.	Test dose, per cent.	Total excretion in μ g.	Test dose, per cent.	Total excretion in μ g.	Test dose, per cent.	Total excretion in μ g.	Test dose, per cent.
D.S., 23 years, 57 kilos.	Vegetarian whole-wheat diet.	1.21	103	8.5	423	6.4	2093	39.8	2638	50.7	2851	55.0	2609	50.1	2732	52.6
B.S., 25 years, 51.5 kilos.	"	1.21	122	10.1	383	5.2	1600	29.6	2701	51.8	2779	53.1	2865	54.9	2947	56.5
R.L., 25 years, 52 kilos.	Vegetarian jowar diet	0.83	113	13.4	714	12	1940	36.5	2638	50.5	2813	54	2798	53.7	2835	52.4
N.R., 31 years 55 kilos.	"	0.83	131	15.7	1032	18	3107	59.5	2991	57.2	3201	61.4	3370	64.8	3246	62.3
R.S., 33 years, 55 kilos.	Vegetarian rice diet	0.91	181	19.8	1253	21.4	2450	45.4	2837	53.1	2912	54.6	2753	51.4	2831	53.0
K.S., 27 years, 57 kilos.	"	1.02*	138	13.5	1010	17.4	1932	35.9	3457	66.5	3551	68.3	3579	68.8	3493	67.1
S.H., 20 years, 50 kilos.	Non-vegetarian whole-wheat diet	1.09	293	27.7	1271	19.6	2047	41.0	2839	51.0	3080	55.7	2903	52.0	2970	53.8
B.R., 20 years, 57 kilos.	"	1.09	273	24.5	1089	16.3	1899	32.5	2940	53.2	3184	58.2	3024	55.0	2880	52.0
N.N., 19 years, 52 kilos.	Non-vegetarian rice diet.	0.94	238	25.3	1120	17.6	2781	50.9	3004	55.3	2862	52.5	2799	51.2	2936	54.0

*In this case subject was given a diet from different stock.

TABLE II.

Showing the effect of substitution of pulse protein by animal protein on riboflavin excretion.

Name of subject and weight	Diet	Period	Protein content of the diet, g.	Riboflavin content of the diet, mg.	Riboflavin excretion in μ g.		Increase in per cent excretion due to change in protein
					Total	As per cent of intake	
A.N., 52 kilos	Rice, pulse, vegetables and fat	P-II	63.9	1.08	155	14.3	9.5
" "	Rice, mutton, vegetables and fat	P-IV	57.4	1.02	247	23.2	
S.H., 50 kilos	Whole wheat, pulse, vegetables and fat	P-II	77.4	1.24	98.5	7.9	17.4
" "	Whole wheat, mutton, vegetables and fat	P-IV	63.8	1.21	293	24.2	
B.L., 57 kilos	Whole wheat, pulse, vegetables, and fat.	P-II	77.4	1.24	122.4	9.8	12.7
" "	Whole wheat, mutton, vegetables and fat.	P-IV	63.8	1.21	273	22.5	

TABLE III.

Showing the riboflavin content of blood of normal male Indians.

Name of the subject	Age	Riboflavin in blood in μ g./100 c.c.
S.H.	20	53
B.L.	20	52
D.S.	23	49
B.S.	25	51
G.J.	23	58
L.C.	21	56
M.D.	25	55
L.T.	27	52

Name of the subject	Age	Riboflavin in blood in $\mu\text{g.}/100 \text{ c.c.}$
H.B. ...	28	51
N.D. ...	31	50
P.T. ...	33	49
R.S. ...	27	52
G.L. ...	22	48
M.K. ...	23	50
R.C. ...	27	60
K.S. ...	30	55
G.P. ...	30	53

11. Study of the quality of proteins, non-proteins and fats of some edible varieties of Bombay fish under Dr. (Smt.) Kamala Sohonie at the Department of Biochemistry, Institute of Science, Bombay.

Ten different varieties of Bombay fishes were analysed for their proximate principles. The moisture, protein, fat, Ca, Fe and P were estimated. It was found that fishes contained a very good proportion of protein, i.e. 15 to 26 per cent. The percentage of fat varied from 0.2 to 2.5 per cent. Fishes were, however, found to be a good source of minerals. The mineral content of fish varied from 0.6 to 2 per cent. Fishes were found to be rich in their phosphorus content. The percentage of phosphorus varied from 200 to 300 mg./100 g. of the edible part of the fish. Halwa, pala, boya and mandeli were found to be rich in their phosphorus contents.

The digestibility of fish (muscle) protein with pepsin was carried out, and it was found that the fish muscle (protein) was very easy to digest. The digestibility of fish-muscle protein is found to be comparable with that of skimmed milk. The isolation of protein from fish muscle and the study of protein is in progress.

The non-protein nitrogen fraction was tested for its different nitrogenous constituents, viz.

1. Total non-protein nitrogen.
2. Total volatile nitrogen.
3. Volatile basic nitrogen.
4. Trimethyl-amine nitrogen.
5. Ammonia nitrogen.
6. Amino-nitrogen.
7. Creatine and creatinine nitrogen.
8. Urea nitrogen.

The non-protein nitrogenous constituents were found to vary to a great extent depending upon the degree of freshness of the fish. This fraction was, however, found to contain a fairly good amount of amino-nitrogen. The detection of amino-acid make-up of this fraction by

paper chromatography showed that it was composed of lysine, histidine, arginine, aspartic acid, methionine, phenylalanine, leucine and isoleucine. The non-protein fraction of prawn (colambi) contained a fairly good amount of proline, whereas it was absent in the rest. Further investigations of this fraction are in progress.

The fat from the edible part of the fish was extracted with ethyl ether dried over concentrated sulphuric acid and was studied from different angles. The nitrogen and phosphorus contents of these fats were estimated. It was observed that the nitrogen content varied from 1 mg. g to 10 mg./g. of fish fat. Shark was found to contain the greatest percentage of nitrogen. The phosphorus content of these fats varied from 0.4 mg. g to 10 mg./g. of fish fat. The ether extract of shark contained the greatest percentage of phosphorus. Pala contained fat which was very poor in its phosphorus content. The N:P ratios of these fats were found to vary from 0.9 to 4.5. It was, however, observed that N:P ratio of shark-muscle fat was the least and that of the pala was the greatest.

The *in vitro* digestibility results showed that boya, bombil, ghol, colambi and shark contained fats which were found to be easily digestible. As compared to the fats of boya and bombil, those of pala, toli and mandeli showed low digestibility. It was, however, found that all the fats from ten different varieties of fishes were very easy to digest. The fats of fishes arranged in order of their decreasing digestibilities (*in vitro*) would be boya, bombil, ghol, colambi, shark, halwa, pala, pomphret, toli and mandeli.

The *in vivo* digestibility of fat was carried out by growth experiments using rice-moth larvæ as test animals. The fish fat possessed a high nutritive value as indicated by good growth of the larvæ. The growth of the larvæ on diets containing fats from different varieties of fish compares favourably with that of wheat fat. The body oils of boya, bombil, halwa, colambi and ghol were, however, found to be superior to wheat fat. The fats of fishes arranged in order of their decreasing digestibilities (*in vivo*) would be boya, bombil, halwa, ghol, colambi, shark, pala, toli, pomphret and mandeli. A good correlation between *in vitro* and *in vivo* methods was obtained.

12. Inquiry on nutritional œdema under Shri N. C. Datta at the Grant Medical College, Bombay.

Fifteen patients clinically diagnosed as 'nutritional œdema' were referred to us for investigation. The 15 cases consisted of 13 male adults, one female and an orphan child six years old. These patients belong to poorer classes, some of them were destitutes picked up by the police from roadside. The age of these patients varied from 26 to 56 years, and the weight from 76 pounds to 104 pounds on admission.

Many of these patients had marked anæmia. The r.b.c. varied from 0.7 to 4.2 million per cubic millimeter, and hæmoglobin from 2 to 12 grammes per 100 c.c. All the patients had œdema of leg and feet. One of them had ascites. Four patients showed signs of mental abnormalities, highly irritable and non-co-operative.

Nitrogen-balance studies were carried out on 10 patients. The protein content of diet had to be gradually increased as many of the patients could not tolerate large quantity of milk or eggs and complained of diarrhœa. Casein hydrolysate had to be given in a few cases to start with.

The period of observation varied from 15 days to two months. It was not possible to follow the cases completely till recovery. The orphan child died after a few observations were made. Three patients became highly irritable and left the hospital against medical advice.

Serum proteins.—The serum protein of all the patients was below normal. The albumin varied from 1.08 g./100 c.c. to 2.44 g./100 c.c. The globulin values were normal in 10 and above 3.0 g./100 c.c. in the remaining 5. One of the patients with globulin above 3.0 g. was finally diagnosed as cirrhosis of the liver and not included in the study.

Volume of the urine.—The daily excretion of urine was low in all cases of nutritional oedema and varied from 450 c.c. to 1,094 c.c.

The total nitrogen excretion in urine and stool.—The total excretion of nitrogen in urine on admission was very low and varied from 1.02 g. to 2.58 g. per day.

The faecal nitrogen during nitrogen-balance studies were within normal range varying from 1.03 g. to 2.87 g. per day.

Results of nitrogen-balance studies.—Five to ten observations were made on patients who could not be followed completely. The patients were in a positive nitrogen balance without significant rise in the nitrogen excretion in the urine during the period of observation showing that nitrogen was retained in the body.

Patient No. 1.—Nine observations were made during 18 days, stay in the hospital. The patient was in positive nitrogen balance with a protein intake of about 42 g. per day. The urinary nitrogen slowly increased from 2.58 g. to 4.02 g. on the tenth day. On increasing the protein to 66 g. the nitrogen excretion increased to 4.75 g. on the 12th day and once to 9.08 g. and then showed slight decrease. The volume of urine increased from 510 c.c. to 904 c.c. on the 15th day. He made clinical improvement and there was definite decrease in oedema. He left the hospital.

Patient No. 4.—This patient could not tolerate more than 1½ pounds of milk per day or more than two to three eggs and complained of frequency of stools and diarrhoea. The protein intake had to be gradually increased. Fifteen observations were made during two months. The volume of urine increased from 884 c.c. to 1,380 c.c. per day with protein intake increased gradually from 23 g. to 51 g. the nitrogen excretion in urine gradually increased from 1.98 g. to 3.27 g. per day. He was in positive nitrogen balance and made clinical improvement. The serum albumin increased from 1.6 to 2.17 when he left the hospital.

Patient No. 5.—A lady admitted with marked anæmia and massive oedema of feet; made marked recovery on high-protein diet. She was available for nitrogen-balance studies at the commencement of this inquiry. At that time her oedema had disappeared. During a period of 21 days with a protein intake of 96 g. daily consisting of milk, milk powder, bread, butter and potatoes. The r.b.c. increased from 3.2 to 4.1 million per cubic millimeter; hæmoglobin from 10.5 g. to 13 g. /100 c.c.; serum albumin from 3.38 to 4.47 per 100 c.c. Her weight on admission was 96 pounds which decreased to 91 pounds when oedema disappeared and rose to 104 pounds when she left the hospital, thus gaining 13 pounds.

Nine observations of nitrogen, balance studies during 20 days showed that the faecal nitrogen was 1.83 g. per day. The volume of urinary excretion per day increased from 1,030 c.c. to 1,530 c.c. She was in a positive nitrogen balance, the nitrogen excretion increasing gradually from 4.2 g. to 10.4 g. and was tending towards nitrogen equilibrium.

Patient No. 7.—A male admitted with marked anæmia and massive œdema and in a very poor state of health ; made remarkable recovery during two months stay in the hospital. He was, however, available for nitrogen-balance studies just 15 days before he left the hospital.

The patient was on a diet consisting of milk, milk powder, meat, egg and the usual hospital diet with a total nitrogen intake of 21 g. per day. The volume of urinary excretion per day varied from 1,530 c.c. to 2,100 c.c. The total urinary nitrogen excretion increased gradually from 15.5 g. to 19 g. per day. The average faecal nitrogen was 1.87 g. per day.

The result indicated that the patient regained normal health with tremendous increase in appetite. The patient attained nitrogen equilibrium at the time he left the hospital. The serum protein reached normal values : total proteins 7.18 g., albumin 4.7 g., and the globulin 2.48 g., per 100 c.c. He regained 23 pounds in weight after the complete disappearance of œdema.

In contrast to this, the nitrogen-balance studies of a case of cardiac œdema showed that he was in nitrogen equilibrium with an intake of about 50 g. of protein and the average nitrogen excretion being 6 g. per day. The total volume of urine excreted was within normal range varying from 1,110 cc. to 1,810 c.c. The total serum protein was 7.6 g. ; albumin 4.07 g. ; globulin 3.53 g. per 100 c.c. This is different from the observations made on 'nutritional œdema' cases.

NITROGEN-BALANCE STUDIES ON NORMAL ADULTS.

Patwardhan *et al.* have reported that the nitrogen excretion in Indian adults is low. We have also observed that the nitrogen excretion from persons of various income group is low. We have started nitrogen-balance studies on five healthy normal persons with usual diet. The results show that the total urinary excretion in these cases varied from 1,230 c.c. to 1,500 c.c. per day. The total nitrogen intake varied from 6.68 g. to 9.44 g. per day. The total urinary nitrogen excreted per day varied from 5 g. to 7 g. per day. These normal individuals maintain a nitrogen equilibrium.

Experiments have to be carried out under more controlled conditions with co-operation of hospital staff and the active co-operation of the patients. Attempts are being made to transfer all cases of nutritional œdema to the Nutrition Ward which is in charge of the Nutrition Officer to the Government of Bombay.

13. Inquiry on the metabolism of fat under Dr. K. P. Basu at the Indian Dairy Research Institute, Bangalore-1.

DETERMINATION OF THE OPTIMUM LEVEL OF FAT IN THE DIET.

In view of the possibility that different fats may possess different optima for growth, it was planned to study the growth of young albino

rats when kept on a complete diet containing some of the commonly consumed Indian fats, such as butter-fat, sesame, coco-nut and ground-nut oils, each of the fats being fed at several levels in the diet.

Experiments were first started with butter-fat. Weanling young albino rats were divided into five groups of eight animals each taking care to have an even distribution of litter-mates and initial body-weight in the various groups. The first four groups were fed butter-fat at 2, 5, 10 and 20 per cent level in the diet. These levels were chosen after due consideration of the range of fat levels in the Indian diets as revealed by the various dietary surveys. The higher level of 20 per cent was also included to find out the possible effect of a high-fat level on the growth. Casein, corn starch, salts (Hegsted, Mills, Elvehjem and Hart, *Jour. Biol. Chem.*, **138**, p. 459, 1941) and vitamins formed the other components of the diet. The composition of the diets fed is given in detail in Table I. The proportion of the calories contributed by the protein to the total calorific value of the diet as well as the ratio of vitamins and salts to the calorific value of the diet was kept constant in all the four groups. The diets were fed in isocaloric amounts, as far as possible, to all the groups. The fifth group served as a control and received the laboratory stock ration. The weights of the animals were recorded every week and the experiment continued for eight weeks.

TABLE I.

Composition of the experimental diets.

Dietary components (per cent)	GROUP NUMBER			
	1	2	3	4
Fat (butter-fat)*	2.0	5.0	10.0	20.0
Cane sugar ...	12.6	12.0	11.0	9.0
Corn starch ...	59.2	55.4	50.0	39.1
Vitaminized starch†	4.0	4.7	5.0	5.6
Casein‡ ...	18.2	18.9	20.0	22.3
Salt mixture ...	3.17	3.77	4.0	4.46
Calories per 96 g. of energy-yielding food	394	409	434	484
Grammes protein per calorie from the above diets	0.0462	0.0462	0.0462	0.0461
Ratio for feeding isocaloric amounts ...	1.102	1.061	1.000	0.897

*Due allowances was made for the moisture content of the butter-fat samples while preparing the diets.

†An alcoholic solution containing the following amounts of vitamins was mixed with 100 g. corn starch, dried below 40° C. and used:—

Thimine 4 mg.; riboflavin 6 mg.; pyridoxin 6 mg.; calcium pantothenate 30 mg.; choline, 3 g.; vitamin A acetate 11.2 mg.; a-tocopherol 44.8 mg.; vitamin D₂ 0.28 mg.; menadione (vitamin K) 4.2 mg.; niacin, 12 mg.

‡Rendered fat-free by extraction for two three-hour periods with boiling alcohol.

The average weekly growth of the animals on the different dietary régimes was compared among themselves. The average growth rate was about 15 g./week/animal in the various groups. Male rats in general showed a higher rate of growth than their female counterparts and among the male rats there was a slight and statistically significant higher growth exhibited by the animals on five and ten per cent fat diets than those on 20 per cent fat diets. Otherwise, all the diets proved equally effective. The relevant data are presented in Table II :—

TABLE II.

Average weekly growth of rats fed various levels of butter-fat in the diet.

Group number	Dietary régime	Average weekly growth	Statistical interpretation
<i>Male rats.</i>			
1	2 per cent butter-fat	17.44 ± 0.5152	1 : 2 N.S. ; 1 : 3 N.S.
2	5 per cent butter-fat	18.05 ± 0.5872	1 : 4 N.S. ; 1 : 5 N.S.
3	10 per cent butter-fat	17.59 ± 0.3127	2 : 3 N.S. ; 2 : 4 S.
4	20 per cent butter-fat	17.19 ± 0.1249	2 : 5 N.S. ; 3 : 4 S.
5	Stock ration ...	18.41 ± 2.1850	3 : 5 N.S. ; 4 : 5 S.
<i>Female rats.</i>			
1	2 per cent butter-fat	13.01 ± 0.8539	1 : 2 N.S. ; 1 : 3 N.S.
2	5 per cent butter-fat	13.25 ± 1.5230	1 : 4 N.S. ; 1 : 5 N.S.
3	10 per cent butter-fat	12.38 ± 1.2060	2 : 3 N.S. ; 2 : 4 N.S.
4	20 per cent butter-fat	12.50 ± 0.5104	2 : 5 N.S. ; 3 : 4 N.S.
5	Stock ration ...	13.00 ± 0.8740	3 : 5 N.S. ; 4 : 5 N.S.

N.S. = Not significant.

The unconsumed residual diets were collected for each animal every day throughout the experimental period and the growth per calorie of intake computed. As will be seen from the data presented in Table III, no significant difference in the growth per calorie of intake of animals kept on the various levels of fat was noticed.

The foregoing preliminary results with butter-fat indicate that so long as the need for the calorie and the vitamins are met, differences in the level of fat in the diet do not exert any significant influence in the growth rate.

EXPERIMENTS WITH SESAME OIL.

Experiments on similar lines as noted above with sesame oil as the fat are nearing completion and the results will be reported in due course.

The diets were renewed every week and kept in cold storage when not required so as to avoid development of rancidity.

TABLE III.

Growth per calorie intake by rats fed various levels of butter-fat in the diet.

Group number	Diet fed	Average calories consumed	Average gain in weight in 8 weeks	Gain in weight per calorie consumed	Statistical interpretation
<i>Male rats.</i>					
1	2 per cent butter-fat	2136	139	0.06520 ± 0.001863	1 : 2 N.S.; 1 : 4 N.S.
2	5 per cent butter-fat	2142	144	0.06733 ± 0.002136	1 : 3 N.S.
3	10 per cent butter-fat	2128	141	0.06616 ± 0.001574	2 : 3 N.S.; 2 : 4 N.S.
4	20 per cent butter-fat	2114	138	0.06504 ± 0.000873	3 : 4 N.S.
<i>Female rats.</i>					
1	2 per cent butter-fat	2022	104	0.05140 ± 0.001899	1 : 2 N.S. 1 : 3 N.S.
2	5 per cent butter-fat	2012	106	0.05257 ± 0.003498	1 : 4 N.S. 2 : 3 N.S.
3	10 per cent butter-fat	1959	99	0.05048 ± 0.003850	2 : 4 N.S.
4	20 per cent butter-fat	1940	100	0.05155 ± 0.001599	3 : 4 N.S.

N.S. = Not significant.

14. Studies on the influence of dietary habits on incidence of goitre and tuberculosis in Darjeeling under Dr. S. R. Sen Gupta, Assistant Director of Health Services, West Bengal, Calcutta.

The work consisted mainly of a clinical survey of goitre amongst both adult and children population in some rural areas near Darjeeling town and a diet survey of the same subjects undertaken simultaneously. Out of the total of 4,214 subjects examined, 1,047 were adult males, 1,082 adult females and 2,085 children of both sexes. By following Ryle's method of classification, it was found that 48.6 per cent of the adult subjects manifested various grades of thyroid enlargement, while 38 per cent of the children examined came under the same category. The pathological condition so far as could be judged clinically was very low, this being about 0.26 per cent, though it is quite likely that a good number

of sub-clinical cases might have escaped. In adults, the incidence of goitre was found to be 70 per cent for females and 32·5 per cent for males, respectively. In children, however, there does not seem to be any significant differences between the two sexes.

Study of the diet consumed by the subjects examined shows that the average diet consumed consists mainly of maize, cabbage leaves, dried and fermented 'Raixak' (the local name of a vegetable, popular and grown locally), bitter gourd, leaves of pumpkin, amaranth and leaves of squash. Locally made Gundruk, i.e. dried and fermented Raisak and fermented Bhutmas, form the frequent and important article of food.

Details of the procedure, adopted data collected, etc :

The work was divided into two parts :

- (i) A clinical survey of goitre, and
- (ii) a diet survey.

(i) *Survey of goitre.*—A clinical examination of the subjects was made in order to study the state of thyroid gland according to Ryle's method and all cases of goitre were classified into four grades according to Prof. Ryle (Ref. M. R. C. Memorandum No. 18, 1948).

Procedure. The clinical method used for grading and recording size and other changes in the thyroid gland is given below :—

The neck was first inspected at rest and then on swallowing, with the head held at a natural angle, the chin being neither unduly raised nor lowered. Swallowing movements helped to satisfy the examiner that an observed fullness did in fact correspond with the gland. The thyroid was then palpated in the region of the lateral lobes with the flat of the finger tips of both hands.

The neck was next inspected in profile ; in this view true thyroid fullness appears as a gently rounded convexity of varying degree, in place of the more usual straight line from the laryngeal cartilages to the supra-sternal notch. Finally, the gland was palpated from behind with the finger tips of both hands.

The state of thyroid gland was recorded in one of the four categories :

- (1) Invisible at rest.
- (2) Visible at rest, but soft, smooth and symmetrical.
- (3) Conspicuously visible, but showing no palpable asymmetry firmness or nodular change.
- (4) More conspicuously visible and showing a degree of fullness asymmetry or nodular change which can be regarded as definitely pathological.

The details of the incidence of goitre in various age groups are shown in the Table.

TABLE.
Summary of results.
State of thyroid gland.

Subjects	Males				Females			
	Grade				Grade			
	I	II	III	IV	I	II	III	IV
<i>Adults</i> ...	708 (67·7)	256 (24·4)	79 (7·5)	4 (0·4)	325 (30·0)	595 (55·0)	158 (14·5)	4 (0·4)
<i>Children—</i>								
14 years ...	70·0 (3·4)	53·0 (2·5)	9·0 (0·43)	25·0 (1·2)	26·0 (1·26)	7·0 (0·33)	1·0 (0·04)
13 years ...	57·0 (2·7)	60·0 (2·95)	7·0 (0·33)	21·0 (0·99)	35·0 (1·70)	5·0 (0·23)
12 years ...	54·0 (2·55)	55·0 (2·60)	6·0 (0·28)	1 (0·04)	30·0 (1·42)	35·0 (1·70)
11 years ...	40·0 (1·90)	46·0 (2·18)	10·0 (0·45)	12·0 (0·57)	30·0 (1·42)	4·0 (0·19)
10 years ...	48·0 (2·20)	58·0 (2·80)	34·0 (1·60)	30·0 (1·42)	3·0 (0·14)
9 years ...	34·0 (1·60)	25·0 (1·20)	2·0 (0·09)	26·0 (1·23)	13·0 (0·6)	2·0 (0·09)
8 years ...	46·0 (2·18)	29·0 (1·30)	25·0 (1·20)	27·0 (1·28)
7 years ...	27·0 (1·28)	19·0 (0·90)	3·0 (0·14)	45·0 (2·10)	25·0 (1·20)
6 years ...	40·0 (1·90)	30·0 (1·42)	34·0 (1·65)	26·0 (1·23)
5 years ...	41·0 (1·95)	18·0 (0·09)	40·0 (1·90)	17·0 (0·80)
4 years ...	51·0 (2·40)	20·0 (0·95)	63·0 (3·0)	22·0 (1·0)	1·0 (0·04)
3 years ...	58·0 (2·80)	11·0 (0·50)	1·0 (0·04)	59·0 (2·90)	17·0 (0·80)	1·0 (0·04)	1·0 (0·04)
2 years ...	85·0 (4·0)	7·0 (0·33)	82·0 (3·90)	5·0 (0·23)
1 year ...	70·0 (3·4)	65·0 (3·09)	1·0 (0·04)

Figures in brackets represent percentages in each case.

(ii) *Diet survey.*—A study of the diet consumed by as many of the individual subjects examined as possible was made. This was done by actually weighing uncooked food daily for a period of seven days. In addition, verbal inquiries were made in all cases regarding the consumption of articles that have a seasonal variation as the present diet survey being carried out in summer and rainy seasons only, failed to supply information regarding the consumptions in winter—which forms the major part and the most taking period of the year. Diet survey was carried out in 123 families in the rural areas near Darjeeling town.

15. Inquiry on physiological study of human breast milk and its correlation with the sub-nutritional status (specially under weight) of the newborn in India under Dr. S. K. Roy and Dr. Amala Ramcharan at the Ramkrishna Mission Sishumangal Prathisthan, Calcutta.

The number of cases observed was 26. Of these six cases were rejected, due to inadequate period of hospitalization.

All babies considered in the survey were full term, normal babies over 5 lb. in weight. As far as was possible, only mothers who were healthy at the time of delivery were considered. The babies were test-fed twice daily starting from the third day of life and milk samples were collected from the mother from the third day on. Wherever possible both fore and after-milk samples were collected. The blood samples from the babies were taken in most cases during the eight-to-ten-day period on three occasions allowing an interval of three to four days between samples.

Maternal history.—Of the 20 mothers investigated, all except three, were Bengali on rice and fish diets. Of the remaining three, two were South Indians on vegetarian diets and one was Punjabi on wheat and meat diet. Their ages ranged from 17 to 39 years and they came from different economic levels, the income per capita varying from Rs. 25 to Rs. 500 or more per month. Of the 20 mothers, 16 were multiparous, one mother having as many as 12 children, the remaining four being primiparous.

Clinical data.—Of the 20 babies observed, ten were males and ten females (*see* Table I), their period of hospitalization varying from 8-14 days.

TABLE I.

Duration of stay in hospital, days		Number of babies
8	...	2
9	...	4
10	...	6
11	...	4
12	...	2
13	...	1
14	...	1

Their birth weight varied from 5 lb. to 8 lb. 5½ oz. (*see* Table II).

TABLE II.

Distribution of babies according to birth weight.

Weight groups	Number of babies
5 lb. 0 oz. to 5 lb. 8 oz. ...	3
5 lb. 9 oz. to 6 lb. 0 oz. ...	5
6 lb. 1 oz. to 6 lb. 8 oz. ...	5
6 lb. 9 oz. to 7 lb. 0 oz. ...	3
7 lb. 1 oz. to 7 lb. 8 oz. ...	3
7 lb. 9 oz. to 8 lb. 0 oz. ...	0
8 lb. 1 oz. to 8 lb. 8 oz. ...	1

Six babies developed a few watery stools which except for one case did not appear to have any significant effect on the weight curve. They were not cases of frank diarrhoea. One child developed a mild form of thrush which did not interfere with nursing at the breast and which disappeared within 24 hours; and another child had talipes. It was not thought necessary to reject the babies with watery stools and thrush though this was one of the conditions under which the work was to be carried out, since these conditions did not appear to interfere with the neonatal progress.

Weight gain.—All babies except six were gaining weight at the time of discharge, but only one attained the birth weight and two surpassed it by a gain of 5·7 per cent and 0·2 per cent of birth weight respectively. The remaining babies lost from 2·1 per cent to 13·9 per cent of their birth weight during the first three to eleven days of the neonatal period. Eight of the 20 children stopped losing weight by the third to fourth day of life, this being within the normal time limit for initial weight loss.

Volume intake.—The milk flow started at the third day post partum in most cases, and the infants were receiving $\frac{1}{4}$ to $\frac{3}{4}$ oz. of colostrum or milk at each of the daily test feedings. Of the 20 babies studied four were receiving $2\frac{1}{2}$ oz. of milk per lb. of birth weight per day by the end of the first week. The others received amounts varying from 1-1 $\frac{1}{2}$ oz. per lb. on the seventh day.

Biochemical data.

Analysis of data collected on infants 'blood and mothers' milk (see Table III).

TABLE III.

Values (ranges) derived from bio-chemical analysis of blood samples obtained from 20 newborn infants during first ten days of life.

Constituents	1st-2nd day	4th-6th day	7th day & after
Hæmoglobin (g./100 c.c.)	15·2-18·26 (15)	12·85-17·75 (15)	13·86-17·75 (14)
Protein (g./100 c.c.)	5·05-6·8 (17)	5·30-6·15 (15)	4·75-6·15 (17)
Albumin (g./100 c.c.)	2·70-3·75 (17)	2·70-4·00 (15)	2·70-4·16 (17)
Sugar (mg./100 c.c.)	35-74 (11)	48-73 (12)	48·82·5 (14)
Calcium (mg./100 c.c.)	8·8-10·1 (6)	8·8-10·55 (8)	8·8-10·00 (11)
Phosphorus (mg./100 c.c.)	4·5-6·0 (6)	5·1-6·6 (8)	5·4-6·6 (9)

TABLE IV.

Values (ranges) derived from biochemical analysis of milk samples from 20 nursing mothers during first ten days post partum

Protein, per cent	Fat, per cent	Sugar, per cent	Calcium, per cent
1.20-1.80 (19)	2.5-4.3 (20)	6.40-7.66 (20)	19-32 (20)

Note.—Figures within brackets in Table III and IV indicate the number of cases. Since the number of cases observed up to date was very small, the above data were not statistically analysed and only the ranges of values have been given.

DISCUSSION.

When comparing the range of average values obtained in this survey (*see* Tables III and IV) with those of other workers, the former tends to be much lower than the latter. The minimum values of the present series are higher, while the maximum values are not as high as the values of other workers. In a comparison between the figures of individual cases and the published mean values, the values of the present survey are found to be on the low side. For example, in the protein values of the mothers' milk (Table IV), neither are the maximum values as high, nor the minimum values as low as the published results—1.2 to 1.8 g./100 c.c. (present series) as compared to 0.66 to 4.86 g./100 c.c. (first to sixth day) and 0.95 to 2.85 g./100 c.c. (seventh to twelfth day) (Gardner and Fox, 1925.)

A similar difference is found in the fat values of milk, that is, the maximum values are not as high, nor the minimum as low as those in the literature—2.5 to 5.3 g./100 c.c. (present series) as compared to 0.56 to 6.54 g./100 c.c. (first to sixth day) and 1.54 to 7.65 g./100 c.c. (seventh to twelfth day) (Gardner and Fox, 1925). A comparison of sugar values shows the same difference—6.4 to 7.66 g./100 c.c. (present series) and 3.00 to 11.90 g./100 c.c. (first to sixth day); 3.80 to 9.20 g./100 c.c. (seventh to twelfth day) (Gardner and Fox, 1925). The published means for calcium varies from 22.4 to 34.4 mg./100 c.c. (Uga, 1935 and Macy, 1949 respectively). In a comparison between published figures on blood samples and the values obtained from this survey (Table III) the following facts emerge :—

1. Maximum values for Hb on first and second days are not as high in this series as those of other workers.

2. Total protein values (present series) are lower after the seventh day.

3. Minimum sugar values are much lower than those in the literature but maximum values are almost the same—first and second day 55 to 75 mg./100 c.c. ; third day 65 to 85 mg./100 c.c. (Ketteringham & Austin). Present series—35 to 74 mg./100 c.c. (first and second day) : 48 to 73 mg./100 c.c. (fourth to sixth day).

Calcium and phosphorus values again show the same trend, i.e. lower maximum values at all periods.

A few interesting facts emerge from a study of the above data in relation to the weight gain or loss of some of the babies :

Case 1.—Male infant, birth weight 6 lb. 7 3/4 oz., second child of a 23 year old mother of good economical status. Baby started to gain by fourth day, and lost 8·6 per cent of birth weight (ten per cent being the upper limit of initial weight loss). At the end of one week baby was getting less than 2½ oz. per lb. of birth weight of milk, and in spite of lower blood albumin (2·7 g./100 c.c.) and very low blood sugar (41 mg. per cent), as well as low protein (1·3 g. per cent) and extremely low calcium (19 mg. per cent) in mother's milk, the infant gained steadily but was removed from hospital on the tenth day before birth weight was attained. The milk flow was well established by the end of the third day. Weight gain occurred in spite of frequent watery stools on the eighth day.

In some children a delay in weight gain has been associated with late milk flow.

Case 2.—Female infant, birth weight 5 lbs. 1¼ oz., fourth child, initial weight loss 8·3 per cent, failure to gain weight, weight curve meandering throughout 13 day period. Milk intake 1½ oz. per lb. of birth weight at the end of the first week. Milk flow late in starting—fifth to sixth day. Fat content of milk high—3·7 per cent gm but protein low 1·2 g. per cent. Other values within normal limits.

Cause of failure to gain perhaps due to delay in milk flow.

Case 3.—This case is an example of many others similar to it, which in spite of early adequate intake of milk ; and blood and milk values within normal limits except for protein 15 g. and calcium 21·7 mg. per cent which are low in almost all cases, there was a lag in the weight curve, the rise beginning after the seventh instead of the third or fourth day as happens in normal babies. This child had no apparent neo-natal illness.

Case 4.—Male infant, 7 lb. 5½ oz. first child, 12 days stay in the hospital, milk intake 2 oz. by end of the first week, Hb. low, 16·65 g. on first day and still lower 12·85 g. on fifth day, blood sugar low, 48 mg. on fifth day. Moreover, baby's initial weight loss was only 4·8 per cent ; began to gain weight by third day and surpassed birth weight by seventh day . No apparent reason for this rapid progress.

COMMENT.

With data available on only 20 cases so far, it is not possible as yet to draw any valid conclusions. However, a review of the above data will reveal some interesting facts regarding the behaviour of the Indian neo-nate during his first eight to ten days of life. Only a proper statistical analysis of the data from 100 to 200 cases will be able to show whether or not the values obtained in this survey is much below the standard. When this analysis is made, it will then perhaps be possible to assess the relationship (if any such exists) between the constituents of breast milk and the nutritional status of the ten-day old infant.

16. Studies on energy metabolism under Dr. S. Banerjee at the Presidency College, Calcutta.

The heat output of the body is usually expressed in relation to the surface area of the body. The surface area of the whole body is, however, universally calculated from a formula suggested by DuBois based on the

height and weight of the person : $A = W^{0.425} \times H^{0.725} \times 71.84$ where A is the surface area in square centimeters, W is the weight in kilogram and H is the height in centimeters. The surface area of the body is sometimes calculated from the linear formulæ of DuBois, where surface areas of different parts of the body are calculated from different formulæ and the sum of the areas of different parts of the body gives the total surface. These formulæ were derived by the actual measurements of the surface area of the body by making paper-moulds in five subjects only. It was considered necessary to find out if the above formulæ would be suitable to determine the surface area of the body of Indians. As heat output is mainly related to the surface area of the body any error in the calculation of the surface area would vitiate the studies on energy metabolism. In the present inquiry attempts have, therefore, been made to determine the surface area of the body by actual measurements in order to find out if the surface area of the body calculated from DuBois formulæ differs from the surface area actually measured.

DETERMINATION OF THE SURFACE AREA OF THE BODY.

Tape method.—Surface area of the body can be measured by pasting narrow, inelastic tapes of uniform width over the entire surface of the body and the area calculated from the length and width of the tape used. In order to find out the reliability of this method in the measurement of the surface area of the body, same parts of the body of the same individual were measured in two occasions and the results compared. It was observed that the results varied only 0.25 per cent. This indicated that the method was quite reliable in the determination of the surface area of the body so far as the cylindrical parts were concerned. It was, however, found inconvenient to measure the surface area of the head, which is irregular, with the tape method. Surface area of the body of four students was actually measured by the tape method and the results compared with the surface-area value obtained from the height and weight formulæ of DuBois. The results are given in Table I:—

TABLE I.
Surface area of the body of persons measured by the tape method.

Subject	Age in years	Weight, kg.	Height, cm.	Surface area as measured by the tape method, sq. cm.	Surface area as calculated from DuBois formula, sq. cm.	Difference in the results by these methods
A. K. S. ...	19	43.2	160.2	15,185	14,120	1,065
A. R. G. R. ...	20	50.9	171.0	16,260	15,880	380
A. K. D. ...	20	41.2	159.9	15,005	13,810	1,195
K. P. C. ...	22	56.5	172.1	16,959	16,690	269

Results showed that the surface area of the body actually measured was more than the area calculated from DuBois height and weight

formula. The two results differed by about seven to eight per cent in two cases, while in the other two cases the results differed by 1·5 to 2·3 per cent. It was, however, not possible to come to any conclusion at this stage regarding the suitability or otherwise of the formula of DuBois before the surface area of the body of a large number of persons is actually measured.

Mould method.—It was not possible to accurately measure the surface area of the head by the tape method as the surface is irregular. It was thought that if a plaster mould of the head could be taken a cast of the head could be prepared out of the mould. From the plaster cast a paper mask could be made from which the area could be measured. Although the process was laborious and tiresome it was perfected after several trials. A tight-fitting rubber bathing cap was used to cover the hair of the head. A paper board with a hole cut according to the shape of the face was tightly fitted. The board rested on the ears so as to divide the head into two almost equal halves. The whole of the face was smeared with cold cream, the eye lashes and mustaches, if present, were covered with cigarette papers, the eyes were protected with a thin layer of dough made of wheat flour. Two rubber-tubes were introduced into the nostrils for the purpose of breathing and the tubes were kept in place by dough. Plaster of Paris cream was prepared with requisite quantity of water and it was quickly poured on the face of the subject who was lying on a table with his face directed upwards. After 15 to 20 minutes when the plaster had set the mould was removed. In the similar way a plaster mould of the other half of the head was prepared. The moulds were dried and the inside portions painted thinly with shellac varnish which was allowed to dry. After this, a thin layer of vaseline was applied. A thin emulsion of plaster of Paris was poured into the mould which was kept in an undisturbed condition for two days to set. After the removal of the plaster mould the plaster cast of the head was obtained and dried. Two halves of the head casts were joined. After the application of vaseline, a thin cloth was fitted on the cast on which small strips of paper were pasted to form an exact mask of the head which was subsequently dried. The mask was removed, cut into small pieces so that each piece could be laid flat. Each piece was either traced on a smooth drawing board or photographically printed. The patterns were cut and weighed. Knowing the area of each gramme of the board, the total surface of the head was calculated from the weight of the patterns. The surface area of the head of two persons was determined and the results compared with the area as determined by the tape method. The results are given in Table II:—

TABLE II.

Surface area of the head as determined by different methods.

Subject	By the plaster cast method, sq. cm.	By the tape method, sq. cm.	By the linear formula of DuBois, sq. cm.
A. R. G. R. ...	1,183	1,090	1,208
A. K. S. ...	1,047	1,137	1,026

Surface intregator method.—Surface areas of the body, except those of the head, fingers and toes can be measured also by the surface intregator. The above instrument is not available in this country. The instrument was devised by us. A revolution counter consisting of a spindle and a round disc containing 100 divisions marked on it was purchased. When the spindle makes one complete revolution the disc moves one division from the index line. To the spindle one sleeve was fitted over which one marking wheel and one knurled wheel of the same diameter was fitted with set screws. Two adjustable clamps were fitted on the handle of the revolution counter to hold a chalk-pencil holder. The chalk pencil was fitted into the holder with a spring behind it so that the chalk pressed on the marking wheel. The surface intregator was rolled over the surface of the body to produce a chalk line on the body surface. The end of the knurled wheel was then placed on the chalk line and the surface intregator was rolled along the first chalk line thereby producing a second chalk line at a distance equal to the distance between the two wheels. From the number of revolutions the wheels made to cover the surface, the circumference of the wheel and the distance between the knurled wheel and the marking wheel the surface area was calculated. The portions of the different parts of the body left unmeasured by the surface intregator were measured by geometrical measurements and thus the total area of the surface was calculated.

Surface area of the body except* the head, hands and feet of two subjects were measured by the surface intregator method. It was observed that it was convenient to measure the surface area of the trunk and thighs with the surface intregator. The parts of the body whose circumference were smaller could be measured with difficulty with the surface intregator. The results are given in Table III :—

TABLE III.

Surface area of the body measured with the surface intregator.

Subject		With surface intregator	With tape method*
A. R. G. R.	...	16,200 sq. cm.	16,353 sq. cm.
A. K. S.	...	14,884 „ „	15,093 „ „

*In both the methods the heads were measured by mould method and hands and feet by tape method.

Surface area of the different parts of the body as calculated from the linear formula of DuBois.—In the linear formula of DuBois, the body had been divided into various anatomical regions, such as head, arms, hands, trunk including neck, thighs, legs and feet. Each of these regions is measured with the help of anthropometric rod and tape in several places to find the average or the characteristic length and circumference of each region. The surface area of each part is calculated from the specific formula given for that part by DuBois. According to DuBois the constants used in the formulæ for arms, thighs, legs, hands and feet when multiplied by the measurements of one side give the surface area of both sides. We have, however, observed that the measurements of these parts on one side of the

body are not always the same as those of the corresponding parts of the other side. This was found true specially in the case of arms. The surface area of different regions actually measured by the tape method and also calculated from the formulæ given by DuBois in two subjects are given in Table IV :—

TABLE IV.

Surface areas of different regions of the body as measured by tape method compared with those obtained by DuBois formulæ.*

Subject	Parts of the body measured	Surface area measured, sq.cm.	Surface area calculated, sq. cm.
A. R. G. R.	Head	1,183	1,208
	Arms	1,152 (R) 1,090 (L)	2,169
	Hands	399 (R) 398 (L)	776
	Trunk	5,673	5,202
	Thighs	1,617 (R) 1,607 (L)	2,411
	Legs	1,029 (R) 1,023 (L)	2,046
	Feet	589 (R) 593 (L)	1,030
Total body surface		16,353	14,841
A. K. S.	Head	1,047	1,026
	Arms	1,037 (R) 1,013 (L)	1,947
	Hands	378 (R) 374 (L)	670
	Trunk	5,417	4,670
	Thighs	1,474 (R) 1,427 (L)	2,024
	Legs	887 (R) 881 (L)	1,994
	Feet	578 (R) 580 (L)	1,006
Total body surface		15,093	13,337

*Head was measured by the mould method.

The preliminary studies on the determination of the surface area of the body as reported above indicate that the work should be continued in a large number of subjects in order to criticize or justify the height and weight and linear formulæ of DuBois.

LEPROSY

1. Leprosy inquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta.

THERAPEUTIC STUDIES WITH ISONICOTINIC-ACID HYDRAZIDE IN THE TREATMENT OF LEPROSY.

Hydrazine derivatives of isonicotinic-acid have been found to be very highly active against tubercle bacillus both *in vitro* and *in vivo*. Of the numerous derivatives investigated, the one found to be most active and well tolerated is isonicotinic-acid hydrazide. The drug has been put to clinical trials in cases of human tuberculosis ; and although found useful, has not fulfilled the expectations first raised about this 'wonder drug'.

The successful use of a drug in tuberculosis always suggests its trial in leprosy. This approach has been more than justified by the introduction in the treatment of leprosy of sulphones and thiosemicarbazones, the two groups of drug first used in the treatment of tuberculosis. The value of this drug in leprosy has, therefore, been investigated.

For this study, two brands of isonicotinic-acid hydrazide received from two sources were used. A supply was first received from Organon Laboratories Ltd., under the name of isonicotinic-acid hydrazide ; and later a supply was received from May and Baker Ltd., through the Secretary, Indian Council of Medical Research, New Delhi, and their product bears the name isoniazid. A group of patients was first put on isonicotinyl hydrazide supplied by Organon Laboratories and later another batch was put on isoniazid. The results in the two batches have been similar, and, therefore, both the groups are covered in one report.

A total number of 31 patients was included in the investigation. Eight of them were in-patients in Gobra Hospital, and 23 out-patients in Leprosy Department of the School of Tropical Medicine, Calcutta. The selection of the patients in the trial was made to ensure the inclusion of the various kinds of lesions and complications, such as reactions, deformities, ulcers, etc. Some cases were included because they had previously been found to be intolerant to other chemotherapeutic drugs (sulphones and thiosemicarbazones).

Of the 31 cases, 7 were of the non-lepromatous (neural) type, and 24 of the lepromatous type. Of the non-lepromatous cases six were untreated, and one had been unsuccessfully treated with streptomycin. Of the lepromatous cases 20 were untreated. Out of these, 13 had different kinds of skin lesions, four had in addition acroteric lesions with loss of sensation, deformities, and ulcers, and three were suffering from lepra reaction. Four of the lepromatous cases had been previously treated unsuccessfully with other drugs—one with streptomycin, two with thiosemicarbazone, and one with DDS. (In the one case treated with streptomycin, the treatment was not effective, and in the three cases treated with thiosemicarbazone and DDS, the drugs were not tolerated and had to be discontinued).

Before starting the treatment the following examinations were completed and the results recorded :—

- (a) Detailed clinical and photographic records of the cases.
- (b) Bacteriological examination of all cases and recording of the results according to Dharmendra's method.
- (c) Lepromin test, using refined lepromin and recording 24-48 hours' readings.
- (d) Hæmatological examination—total and differential counts, hæmoglobin estimation, mean corpuscular volume, etc.
- (e) Liver-function tests, including Vandenberg and thymol-turbidity tests.
- (f) Urine for routine and for urobilinogen.

The above examinations except the lepromin test were repeated at regular intervals and the results compared with initial findings.

A study was made of the absorption and excretion of the drug to get an indication regarding the dose and interval. It was observed that after oral administration, the drug is rapidly absorbed and that the maximum concentration in the blood is reached between two and six hours after administration. It is quickly excreted and disappeared from the blood within 16 to 24 hours, mostly through urine. The maximum concentration in blood after an intake of 100 mg. is about 0.5 mg. per cent, four hours after administration. With 200 mg. twice daily the maximum concentration is about 0.8 mg. per cent which comes down to 0.2 mg. per cent at the end of 12 hours.

Dosage.—The initial dose in most cases was 50 mg. per day, except in five cases who had 25 mg. per day as they were suffering from reaction or were known to be intolerant to other chemotherapeutic drugs. Except in these five cases, the dose was increased to 200 mg. given in two divided doses of 100 mg. each. When no appreciable clinical improvement was found in about five months, in 12 cases the dose was further increased to 400 mg. (200 mg. twice daily) to see if a higher dose would be more effective.

Period of treatment.—Three cases, all of the lepromatous type, took treatment for less than four weeks, one of them developed reaction on even small doses of I.N.H., isonicotinic-acid hydrazic) and the treatment was discontinued after the first week. The other two were suffering from reaction when put on I.N.H. and because no improvement was noticed they discontinued the treatment. In the remaining 28, treatment was continued from 14 to 57 weeks. The average duration of treatment was 42 weeks in case of the non-lepromatous cases, and 35 weeks in case of the lepromatous cases.

Tolerance.—The drug is on the whole well tolerated in daily doses up to 400 mg. (5 mg. to 7 mg./kg.). There is slight initial fall in r.b.c. and Hb., but this is restored in all cases, and in several the readings are even higher than those before treatment. No evidence of liver damage has been observed. However, some of the lepromatous cases who did not, tolerate sulphones and thiosemicarbazone, did not tolerate I.N.H. as well.

Therapeutic effects : Non-lepromatous (neural) cases.—Of the seven non-lepromatous (neural) cases, there was seen clinical improvement in six in the shape of subsidence of the patches after treatment for about two

months. Later (five months to a year) the activity of the disease, however, increased even in spite of an increase in dose. There was no change in the extent of anæsthesia. In the remaining one case, there was no improvement whatsoever, this was the case which had been previously unsuccessfully treated with streptomycin.

Lepromatous cases.—Of the 24 lepromatous cases four were suffering from reaction, three were known to be intolerant to other drugs, and 17 did not have any such history or complication.

None of the cases with reaction, derived any benefit, and treatment was suspended in one case after one week and in three cases after 4 to 14 weeks. The three cases that were intolerant to other drugs (sulphones and thiosemicarbazone) were also found intolerant to I.N.H. and treatment had to be suspended in one case after one week and in the other two cases after 14 weeks. The dose in these cases had to be kept low (25 mg. to 50 mg. per day) and there was no improvement in any of them.

Of the remaining 17 cases, slight to moderate clinical and bacteriological improvement was seen in 15 cases after treatment for two or three months, and in two of them the improvement was very definite and marked. The bacteriological improvement was, in general, more marked than usually seen with sulphones and thiosemicarbazones in a similar period. The clinical improvement included subsidence of the patches and infiltrated areas, but there was no improvement in acroteric lesions (loss of sensation, deformity, ulceration, etc.) in the cases which had such lesions. In one case with multiple nodules, the nodules broke down and were followed by healing and scarring. However, as the treatment was continued there was seen a set back, both in clinical and bacteriological conditions in all the cases (except one) that had shown initial improvement, in spite of the fact that the dose had been gradually increased. The one case in which deterioration was not seen till up to a year's treatment has also started showing bacteriological deterioration as judged by the last and recent examination. The set back was manifested in the increase of erythema and thickness of lesions, appearance of fresh lesions, new involvement or further extension of old involvement of mucous membrane and increase in bacteriological index. In four of the 15 cases which had shown initial improvement, the set back in later months took the shape of lepra reaction.

DISCUSSION.

The results reported would tend to show that while I.N.H. has some action on the leprosy bacillus in the beginning, possibly the bacilli become resistant to I.N.H. early (8 to 12 weeks) in the course of treatment. This is in contrast to the experience with sulphones and thiosemicarbazone in which no such indication of development of resisting strains of leprosy bacilli have been found. In this connection it would be interesting to note that workers in tuberculosis are also finding the development of resistant strains of tubercle bacilli after comparatively short periods of treatment with I.N.H.

Our results are in conformity with those of Lowe who did not find any significant improvement with I.N.H. We did not, however, find any beneficial effect in cases of reaction in which he has found the drug of some value. Our findings do not agree with the finding of workers who

have reported extremely beneficial results with I.N.H., although dosage (400 mg./day) in 12 cases was comparable to the high doses (5 mg./kg. to 7 mg./kg.) used by some of them. This difference in results may be due to the fact that most of these workers have reported on the results obtained in the first two or three months. According to our experience the early beneficial results seen in the first two months of treatment with this drug are not maintained on further continuation of the treatment, even when the dose is increased. As explained earlier this may possibly be due to the early development of I.N.H.-resistant strains of the leprosy bacillus.

CONCLUSION.

It may be concluded that I.N.H., though found of some value in the first eight to ten weeks, is not very effective in the treatment of leprosy. Neither has it been found of value in the treatment of acute or sub-acute lepra reaction. It is possible that in combination with some other anti-leprotic drug, I.N.H. may be of some value in the treatment of leprosy. If our assumption regarding the development of resistant strains of leprosy bacilli to I.N.H. early in the course of treatment be correct, it would indicate the need for combining I.N.H. with some other anti-leprotic drugs (sulphones or thiosemicarbazones) from the start, or for changing over from I.N.H. to one of these drugs after about two months of treatment with I.N.H. during which initial improvement will be noted in most cases.

THIOSEMICARBAZONE.

Studies on p-acetylaminobenzaldehyde thiosemicarbazone have been continued on 66 lepromatous and 19 neural cases. Of these 54 lepromatous and 19 neural cases were on thiosemicarbazone alone, and the remaining 12 lepromatous cases received combined treatment of sulphone and thiosemicarbazone. The duration of treatment with 'Siocarbazone' varied from 85 to 135 weeks with an average of 115 weeks, and with 'Neustab' (or thiacetazone) the duration of treatment was from 18 to 95 weeks with an average of 65.5 weeks. The combined group received treatment on an average 61 weeks the maximum being 113 weeks and minimum 17 weeks. Last year's dosage scheme and procedure of administration have been followed during this year.

The results obtained up to the preparation of this report have confirmed the findings of last year. The clinical improvement have been noticed in all cases except four who have not been able to tolerate more than 50 mg. of the drug. Gradual disappearance of lesions, restoration of sensation in anæsthetic parts, growth of new hairs in depilated areas and regenerative changes in nails have been noticed. In many cases there is reduction in size of the thickened nerves and marked improvement of tenderness and pain. Extensive acroteric anæsthesia of long duration has not, however, shown any appreciable change. Lepromatous lesions have shown marked regression in most of the cases. In some cases lesions have almost completely subsided. Of the 66 lepromatous cases 11 have become bacteriologically negative. Marked bacteriological improvement has been observed in 16 cases, slight to moderate improvement has been noticed in 24 cases, 11 cases have remained more or less stationary and the remaining four cases showed slight deterioration of bacteriological

index. On the whole, it has been noticed that the bacteriological improvement is of the same order as seen in cases under sulphone treatment though it is not so rapid in the beginning. The following is the summary of the progress under thiosemicarbazone treatment :—

1. *Neural cases* 19
 - (a) Patches disappeared in four cases.
 - (b) Complete restoration of sensation in anæsthetic areas in four cases.
 - (c) Erythema and thickening subsided in eight cases.
 - (d) Return of pigment in hypopigmented patches in four cases.
 - (e) Partial return of sensation in affected parts in 15 cases.
 - (f) Growth of new hairs in two cases.
2. *Lepromatous cases* 66
 - (a) Lesions subsided and smears became negative in 11 cases.
 - (b) Marked clinical and bacteriological improvement in 16 cases.
 - (c) Definite clinical improvement but slight to moderate bacteriological improvement in 24 cases.
 - (d) Some clinical improvement but no bacteriological improvement in seven cases.
 - (e) No clinical and bacteriological improvement in four cases.
 - (f) Clinically and bacteriologically worse four cases.

p-AMINOPHENYL SULPHONYLBENZALDYDE THIOSEMICARBAZONE.

Chemical and pharmacological studies and toxicity tests of the drug have been completed. Bacteriostatic activities of the drug have also been tested. The drug has been found to be well tolerated by animals up to 15 mg./kg. of body-weights. A single dose of 6 mg./kg. given orally or intramuscularly produced no toxic manifestations in men. The pharmacological study included absorption and distribution of the drug in different body fluids and tissues of animals, its rate of excretion through urine and maintenance of the drug in the system. Absorption and excretion of the drug in human being has also been studied. The drug is almost equally distributed in different tissues and body fluids except in adrenaline glands where the concentration of the drug is about double that of the blood and other tissues. Excretion through urine accounts for about 55 to 76 per cent of the drug. The maintenance of the drug in the system has also been studied. It has been noticed that after a single intramuscular injection of 10 mg./kg. dose in rabbit the drug could be detected in urine for about nine days. In men after a single 4 mg./kg. oral dose the drug was detected for about six days but in case of intramuscular administration the drug was detected in urine for ten days.

*The bacteriostatic value of the drug against Kedrowsky's bacillus is not of high order. Only one in 10,000 dilution incorporated in media

could inhibit the growth of the organisms for 12 days. As regards bacteriostatic activity against *Strepto. hæmolyticus* one in 20,000 dilution inhibited the growth of the organism. As the drug breaks down in the system into its components (i.e. thiosemicarbazone and sulphone) and as the bacteriostatic activity against acid-fast organism was found to be of low order, further experiment with this drug in the treatment of leprosy has been abandoned.

DI-HYDROCARPYL-DI-AMINO DI-PHENYL SULPHONE.

The preparation was synthesized by A. K. Bose and A. B. Cattanaach of Messrs. Smith Stanistreet & Co., Ltd., Calcutta, and supplied for trial. The preparation is a synthetic combination of hydnocarpic acid and di-amino di-phenyl sulphone. Toxicity test has shown that the drug is non-toxic even at 25-mg./kg. dose given to rabbit intramuscularly daily for more than two months. The drug is slowly excreted through urine. It seems that the drug does not disintegrate into its components in the system. Bacteriostatic activity of the drug is of the same order as that of p-aminophenyl sulphonylbenzaldehyde thiosemicarbazone. Thirty-five patients (both lepromatous and neural) have been put under this drug. It is yet too early to comment on its effect in leprosy. Some French workers, however, reported beneficial results in leprosy with a similar product derived from the synthesis of di-amino di-phenyl sulphone and chaulmugric acid.

ORTHOHYDROXY DERIVATIVES OF DI-AMINO DI-PHENYL SULPHONE (DIOXYPHONE).

The drug has been synthesized by Dr. H. G. Biswas of Bengal Chemical & Pharmaceutical Works Ltd., Calcutta, and supplied for clinical trial. The toxicity test in guinea-pigs has shown definite toxic manifestation with the oral dose of 20 mg./kg. of the drug. Twelve mg./kg. dose has been fairly tolerated by the guinea-pigs. Bacteriostatic activity against Kedrowsky's bacillus showed complete inhibition of the growth of the organism with a 1 in 100,000 dilution but its bacteriostatic activity against *Strepto. hæmolyticus* found to be poor. One in 10,000 dilution has partially inhibited the growth of the organisms. Its high bacteriostatic activity against acid-fast organisms, however, justified its trial in leprosy and accordingly the therapeutic trial has been arranged and will be commenced shortly.

ACHYRANTHES ASPERA.

Shri R. P. Srivastava from Haldwani had reported marvellous results in the treatment of leprosy with ethereal extract of *Achyranthes aspera* leaves. The treatment was to be given only for ten days and he reported healing of leprosy ulcers and restoration of sensation within a week. Beneficial result was reported in leprosy of all types and in Shri Srivastava's experience there had been no relapse of the disease during the last two years. On reference from the Secretary, Indian Council of Medical Research, trials with this remedy were undertaken in order to find out if the plant really possessed therapeutic activity against leprosy.

A reference was made to the literature to find out the medicinal value of this preparation. It was ascertained that *Achyranthes aspera* is a well-known medicinal plant. In his book on indigenous drugs, Col. Chopra mentioned its use as a purgative and diuretic. In the 'Indian Medicinal Plants', Kirtikar and Basu state that 'the plant possesses valuable medicinal properties as a pungent and laxative and is considered useful in dropsy, piles, boils, eruptions of the skin, etc.' It is also stated that in Ayurveda it is considered useful in leprosy. In different parts of the world the plant is said to have been used for a variety of ether ailments, such as asthma, cough, rheumatism, dysentery, ulcers, warts, and for persons bitten by rabid dogs. 'Every part of the plant is recommended in the treatment of snake-bite (Charaka, Sushruta, Vagbhata, Yogaratnakara, Roberts) and scorpion-sting (Charaka, Sushruta, Subodhavaidyaka), but no part has been found effective in the antidotal and symptomatic treatment of either snake-bite (Mahaskar and Caius) or scorpion-sting (Caius and Mahaskar).

Supply.—In order to be sure of the genuineness of the supply leaves of *Achyranthes aspera* were obtained from Shri Srivastava. He sent them in several instalments by parcel post. Later, the supply was supplemented to some extent from local source after being sure about the genuine nature of the plant.

Preparation of the extract.—Ethereal extract was prepared according to the directions of Shri Srivastava. Leaves of *Achyranthes aspera* were packed in a glass-stoppered bottle to which *spt. ætheris* was added to fill the bottle. The bottle was then tightly stoppered and well secured to prevent evaporation and then kept at room temperature for five days with occasional stirring. At the end of the period, the extract (dark-green liquid) was transferred to another glass-stoppered bottle for use.

Dosage.—The following resume of dosage as advised by Shri Srivastava has been followed. Patients were directed to take the medicine on empty stomach, morning and evening, mixed with one ounce of water :—

Morning	6.00 a.m.	60 drops of the ethereal extract in an ounce of water					
	6.20 a.m.	"	"	"	"	"	"
Evening	6.00 p.m.	"	"	"	"	"	"
	6.20 p.m.	"	"	"	"	"	"

Period of treatment.—According to Shri Srivastava the treatment was to be given only for ten days. Since no improvement was noticed after ten days and since this period is considered to be greatly inadequate for obtaining any results in leprosy, the treatment was extended up to four weeks. It was started on 29-7-53 and was continued till 25-8-53. Before starting the treatment a careful record was made of the symptoms of the patients and of bacteriological and hæmatological findings. During the treatment cases were seen twice a week and at the end of four weeks the bacteriological and hæmatological examination were repeated.

Number and type of cases.—A start was made with ten patients, but one of the patients had discontinued to attend, so the trial was made on nine patients. Of these nine patients, seven were of the lepromatous type and bacteriologically positive, and two of the non-lepromatous (neural) type and bacteriologically negative.

Of the two non-lepromatous cases, one had a few tuberculoid lesions and the other had a few flat hypopigmented lesions (maculo-anæsthetic) with acroteric type of anæsthesia in hands and feet, atrophy of small muscles of both hands and deformity and trophic ulcers under the right great toe.

Of the lepromatous cases, besides the usual skin lesions, two had such complications as ulcerated nodules, acroteric anæsthesia and trophic ulcers in one case.

Results.—Most of the patients reported a feeling of well-being and improved appetite, specially in the first week. A few complained of looseness of bowels, and one of a feeling of drowsiness and lethargy.

Of the two non-lepromatous (neural) cases there was seen no improvement regarding the subsidence of lesions, anæsthesia, deformity, or the healing of trophic ulcers.

Of the seven lepromatous cases one became definitely worse as in this case there was increase in nodulation and infiltration. In four there was no change whatsoever in the leprous condition anæsthesia, deformity, or leprous ulcers though in one case a septic trophic ulcer of recent origin healed in three weeks. Slight improvement as judged by the subsidence of infiltration and thickening was seen in the remaining two cases.

In none of the seven cases was any bacteriological improvement seen. There was no deterioration in r.b.c. or hæmoglobin values. No other toxic symptoms were seen except the feeling of drowsiness and lethargy reported by one case.

Conclusion.—The above investigation has not confirmed the claims of Shri Shrivastava regarding the rapid cure of all kinds of leprous lesions, during a course of ten days treatment with *Achyranthes aspera*. Considering the gross inadequacy of the suggested period of treatment it was extended to four weeks. Even after this period no definite improvement was seen ; in only two of the nine cases was there indication of some clinical subsidence. It is, however, difficult to say whether it was a mere coincidence or caused by the treatment. No bacteriological improvement whatsoever was seen. It may, however, be said that four weeks is too small a period to expect any definite clinical or bacteriological improvement in cases of leprosy.

THE POSSIBLE RÔLE OF B.C.G. VACCINATION IN PROPHYLAXIS AGAINST LEPROSY.

As reported previously, investigation along three lines was suggested : (1) to verify the report of other workers regarding the effect of B.C.G. vaccination in changing a negative lepromin reaction to a positive one, (2) to find out how long this induced lepromin positivity persisted, and (3) to investigate whether the increased sensitivity to lepromin confers any protection against leprosy. Investigation along the first line had already been undertaken and the findings reported, confirming the positivating effect of B.C.G. vaccination.

During the current year, investigation along the second line was undertaken as was proposed last year. It was found that the B.C.G.-induced lepromin positivity, in healthy non-contacts, persisted up to one year, and also that a higher percentage of persons was found to be lepromin positive, one year after the B.C.G. vaccination, than after three months.

This was in marked contrast to the tuberculin reaction in which the percentage of positivity was much lower one year after the vaccination than after three months.

2. Leprosy inquiry under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

HISTOLOGY OF LESIONS IN LEPROSY.

1. Study of early macular lesions of leprosy.

During the last year macules in general were studied. This year attention was solely devoted to the early macular lesions which are found to occur in *contacts of leprosy patients**. Such lesions appear as flat hypopigmented areas (rarely erythematous) and usually fairly well defined. They show no sensory disturbances, or because of the young age of the patients these changes cannot be elicited.

These hypopigmented lesions are differentiated from a host of conditions other than leprosy because they have always been found positive for acid-fast bacilli by concentration methods. Hence, an elucidation of their structure was undertaken. Such early hypopigmented macular lesions from 53 persons were biopsied and a careful histological study made in 30 of them so far.

In five cases, routine sections revealed no change from the normal ; no inflammatory cells being seen except for a slight invasion of the epidermis in certain places by tissue histiocytes. In three of these cases, however, stray bacilli were found in the interstitial tissue or cell cytoplasm.

Twenty out of the remaining 25 cases showed a mild proliferation of histiocytes (round cells) around the blood vessels—both in the superficial and deeper layers of the dermis, together with a few plasma cells. The nerves were uninvaded ; neither were any cells seen around them except in one case. Stray bacilli were revealed in nine of the twenty cases after a prolonged search and (what is more important) in three cases in an intra-dermal nerve twig as well.

In the remaining five cases commencing epithelioid cell change was observed with invasion and fraying of one or two nerves.

These findings are summarized in the Table :—

TABLE.

Serial number	Number of cases	Histology	Number of cases +ve for bacilli in sections
1.	5	No change from the normal	3
2.	20	Perivascular proliferation of histiocytes and few plasma cells.	9 (also in nerves in 3 of these)
3.	5	Histiocytes ; few epithelioid cells slight nerve damage.	2
Total number of cases	30		Bact. +ve 14 cases.

*Figueredo and Desai ' Leprosy in Children ' Ind. Jour. Child. Hlth. 1 pp. 285-295, 1952.

From the above Table it will be observed that the cellular reaction in the majority of these early lesions is scanty and not characteristic or specific of leprosy. The sections stained for bacilli, however, revealed acid-fast organisms in a large number of cases (14 out of 20), besides being detected in the nerves as well in three of them. The unspecific histological structure of these lesions, did not, however conform to that of either of the well-established types of leprosy, viz. the lepride (tuberculoid) or lepromatous forms. This was in fact expected at such an early stage of the disease and was taken to indicate a commencing pathological change in the skin as a result of the presence of the micro-organisms within the tissues. Histologically, at least one could say that these lesions were truly 'indeterminate', to apply a common term in leprosy. As noted above in five cases, commencing (slight) nerve damage was, however, seen indicating that some of these lesions were evolving towards the lepride type.

In a follow-up of these patients one case so far has developed a typical well-established lesion of the lepride type. None have developed any lesions of the lepromatous type.

II. *Continuation of the study of the histological changes observed in two groups of lepromatous patients under the effect of DDS and thiosemicarbasone ('Neustab'), respectively.*

At the outset, it must be noted that no significant differences in the effects of these two drugs was observed. What follows, therefore, applies to both groups of patients. Because of irregular attendance in taking treatment, only 16 out of 35 patients who were due at the six-monthly interval after commencement of therapy could be biopsied at the appointed time. For the same reason, three out of four patients due after one year's and two out of four patients due after 1½ years' treatment, respectively, were biopsied. The findings at these three intervals are given below.

1. *After six months treatment.*—In these cases clinical improvement was not seen or was slight. Corresponding histological changes were also very mild. The inflammatory exudate was not appreciably diminished in amount. The most noticeable change was the marked increase in the vacuolation of the cells in all the cases. This was expected and is usually seen when the growth and multiplication of the bacilli is on the decline due to unfavourable conditions. A considerable thickening of the intercellular collagen framework was observed, which with special connective tissue strains was seen to surround each individual lepra cell. There was no noticeable decrease in the number of bacilli. Their arrangement in the cells was, however, altered. Fewer tightly packed bunches were observed compared with the biopsies before treatment. The majority of them were arranged loosely around the vacuoles of the cell cytoplasm. Further, whereas the most common forms of bacilli were in the shape of straight and curved rods before treatment at this interval, beaded and dumbel shapes predominated.

2. *After one year's treatment.*—Out of the three patients one patient (moderately advanced) was from the 'Neustab' group and other two (advanced cases) were from the DDS group. In the former, the clinical improvement was marked, the few nodules had flattened out and the infiltrated areas were hazy and difficult to locate. In the latter the subsidence of infiltration was not so marked. The histological changes in both groups

of patients were similar to those at the six-month interval but were seen to a greater degree with a considerable decrease in the cellular exudate. A definite diminution in the number of bacilli was also observed with a central clearing of the bacillary masses in the globi.

3. *After one-and-a-half year's treatment.*—One patient was under DDS and the other under 'Neustab' treatment. There was a complete subsidence of the infiltrations in both patients. The flattened areas appeared wrinkled as well. Loss of erythema was, however, observed only in the patient under DDS. In the sections the cellular exudate was markedly reduced compared with the biopsy before treatment. The number of bacilli also were substantially reduced in number. Some of the lepra cells showed only a few bacilli which were understained. No granular forms were seen.

From the above study, so far, it will be seen from the histological changes that the effects of these two drugs is a very gradual process. These patients are being followed up at six-monthly intervals as usual.

3. Leprosy inquiry under Dr. Paul W. Brand at the Christian Medical College, Vellore.

This is the third report on the work of the Leprosy Inquiry at the Christian Medical College, Vellore, and represents the completion of 2½ years of work on surgical aspects of leprosy. It is now possible to report on some tentative conclusions concerning the operative procedures which have been tried out and which are recommended for the reconstruction of hands that have been damaged by leprosy. The foundation for the operative work has been laid by the extensive surveys on some thousands of leprosy hands in which it has been shown that the paralysis caused by leprosy is very strictly limited in its extent and involves almost always the same groups of muscles. The electrical-stimulation tests which form the basis of part of this work has been summarized by Miss Ruth Thomas, S.R.N., M.C.S.P., who completed her work on this inquiry in June this year.

The conclusions of the clinical assessments have been summarized in a Huntarian Lecture delivered by Dr. Brand before the Royal College of Surgeons in London. This was published in the *Annals of the Royal College of Surgeons* and reprinted by the *Leprosy Review*.

The most common paralysis in leprosy is that of all the ulnar-supplied intrinsic muscles of the hand (lumbricals, interossei, abductor and flexor brevis of the thumb and abductor and opponens of the little finger, also the flexor carpi ulnaris). The next most commonly paralysed muscles are the intrinsic muscles of the thumb supplied by the median nerve and the lumbricals to the index and long finger, also the ulnar-supplied flexor profundus muscles.

Much more rarely paralysed are the extensors of the wrist and of the fingers and the thumb. If any of the extensor group is paralysed it is likely that they will all be paralysed.

The muscles that appear to be immune or nearly immune from the paralysis of leprosy are the flexor sublimis of all the fingers, the flexor carpi radialis, the palmaris longus and the flexor profundus to the index

and long finger and the flexor longus of the thumb. Those are the muscles which can be recommended for use in tendon transplantation operations.

The following operations have been used, some of them a few times and some of them on several hundred occasions for reconstruction of deformed hands. Comments on each procedure are appended. Operations that have been modified and adapted from existing operations are marked with an asterisk. Operations which have been developed entirely in the course of this inquiry are marked with a †. Operations devised by other surgeons for other conditions and used in this Inquiry for leprosy are unmarked.

Details of these operations are not included because they would take up a very considerable amount of space, but during the coming year it is proposed to publish them fully in the *Indian Journal of Surgery* and in two numbers of *Bone and Joint Surgery*. A request has been received for a section on the Reconstruction of Hands in Leprosy for the Annual Volume of *British Surgical Practice* and for the *American Year Book of Orthopædics*. After a little more experience in assessing the results of these operations it should be possible to publish a Hand-Book of Surgery for Leprosy which will incorporate the results of our experience in both hand surgery and surgery of the feet and physiotherapy procedures, for the use of medical officers in leprosy sanatoria and orthopædic surgeons who may act as visiting surgeons.

*1. *Sublimis transplantation devised by Bunnell (modified at Vellore).*
Indications.—Paralysis of intrinsic muscles of the hand causing claw-hand deformity in the fingers. Suitable for cases in which the flexor profundus is in good condition and in which the interphalangeal joints are mobile or can be made mobile by exercises and physiotherapy. It is not suitable for cases in which fingers cannot be passively opened out to an angle greater than 100° at the proximal interphalangeal joint.

Method.—In each finger, through a mid lateral incision, the sublimis tendon is detached from its insertion. It is pulled out into the palm and threaded down through the lumbrical canal and sutured to the lumbrical insertion and the dorsal extensor expansion. Thus, the muscle which was used previously to flex the finger joints now acts as an extensor.

Results.—This operation has been extensively used at Vellore on some hundreds of cases and produces very satisfactory results. It can be thoroughly recommended for the majority of claw-hands in leprosy. The operative procedure has been considerably simplified as a result of the experience of the past $2\frac{1}{2}$ years, so that it is now possible to operate upon all four fingers of the hand within about one hour.

†2. *Skin grafting on fingers (devised at Vellore).*

Indications.—For advanced cases of claw-hands in which continued disuse has caused a permanent contracture of the finger joint which cannot be passively opened. This operation is not suitable for cases in which there is bony joint damage.

Method.—A very large number of different methods have been attempted and discarded for this difficult procedure. It is not difficult to open out the fingers and graft the skin gap, but it is very difficult to do so without causing severe post-operative stiffness in extension. The method now recommended is to cut a square sliding flap of the volar half of the skin

of the finger and let it slide distally as the finger joint is opened. When the finger is straight, if the flap has been properly placed, it will be found that the flap lies in front of the interphalangeal joint and that there is a raw area proximal to the joint. This raw area is then covered with a full thickness Wolff graft from the arm. The significance of this procedure is that it allows the joint itself to be entirely surrounded by normal skin, while the graft area comes in the shaft of the finger.

Results.—Stiff fingers are always difficult to treat, but this procedure has given better results than any other used so far.

3. *Interphalangeal arthrodesis.*

Indications.—For clawed fingers with extreme flexion contracture accompanied by bony joint deformity.

Method.—The fingers are opened out forcibly to the desired angle and the joint cartilages chiseled off until they fit in the new position. A fine stainless steel Kirshner wire is drilled from the proximal phalanx into the second phalanx to maintain the angle. A split skin graft is used to fill the skin defect on the front of the finger. The angle recommended for fusion is 145° for the index finger (or the best angle to meet the thumb in a pinch) 100° for the long, ring and little fingers (the best angle for a grasp against the palm). In cases where the cosmetic effect is more important to the patient than the use of the hand, an angle of 150° may be used for all fingers.

Results.—Interphalangeal arthrodesis gives the possibility of a strong grasp by using the metacarpo-phalangeal joints and allows the hands to appear more normal. It only gives a limited range of usefulness.

4. *Extensor proprius transplantation (devised by Fowler).*

Indications.—Intrinsic paralysis giving rise to claw-hand. This operation is probably suitable for cases with a mild degree of deformity and may be suitable for fingers in which the profundus is weak.

Method.—The extensor indicis proprius and the extensor digiti minimi muscles are used for transplantation. Each tendon is split into two and one-half used for each of the four fingers. The tendon slip is passed from the dorsum of the hand through the interosseus muscles to appear in the finger-web anterior to the transverse metacarpal ligament. It is then sutured to the lumbrical tendon in the same way as in Bunnell operation.

Results.—Personal experience with this operation has been extremely limited and it is not possible to express an opinion on its efficacy at this stage.

†5. *Check-rein operation (devised at Vellore).*

Indications.—The 'Intrinsic plus' hand. Cases in which, previous to paralysis of the intrinsics, or in place of paralysis of the intrinsics, there has been a spasm of these muscles producing over-action. If this spasm is long continued it may produce permanent deformity of hyper-extension at proximal interphalangeal joint and flexion at the terminal interphalangeal joint. This operation may also be used for cases in which the 'intrinsic plus' deformity has been produced by a sublimis transplantation operation.

When this operation has been performed on very mobile fingers, it may produce an over-correction of the claw-hand deformity. It is now being used routinely in conjunction with the Bunnell's sublimis transplantation in all cases where there is a hyper-mobility of the interphalangeal joints pre-operatively.

Method.—A free tendon graft is placed across the anterior aspect of the interphalangeal joint. It is sutured distally to the volar edge of the lateral collateral ligament of the interphalangeal joint and proximally to the edge of the digital vaginal ligament. Where the operation is performed as a part of the Bunnell sublimis transplantation, the distal stump of the sublimis tendon itself may conveniently be used. In this case the insertion is left undisturbed and the proximal end of the stump is sutured to the digital vaginal ligament with the finger just short of hyper-extension.

Results.—This operation has only been performed for the past few months, but it seems to have been successful in all cases in preventing the deformity.

6. *Abductor-opponens replacement for the thumb.

Indications.—Intrinsic paralysis of the thenar muscles. A thumb which is unable to oppose the fingers in a grasp or pinch. This operation has a very wide application and it is quite exceptional to find any thumb so badly damaged that it cannot be repaired.

Method.—A number of different operations have been tried for this and have now all been discarded in favour of a modification on an operation described by Riorden of New Orleans. We have further modified this operation in order to produce the best possible range of grasp and pinch. The sublimis tendon from the ring finger is usually used. If the profundus tendon to the ring finger is weak, the sublimis tendon from the long finger may be used. If a sublimis transplantation is used for the fingers, the lack of a spare tendon for the thumb may be made up by splitting one of the other tendons into two and using a half for the long finger and half for the ring. The sublimis tendon for the thumb is detached from its insertion in the finger and is pulled out through a small incision in the forearm. It is then passed subcutaneously through a tunnel to the middle of the wrist and then subcutaneously across the thenar eminence to appear dorso-laterally on the thumb. It is then passes through two slits in the capsule of the metacarpo-phalangeal joint half way between the insertion of the abductor brevis and the extensor longus tendons and is inserted into the extensor longus tendon of the thumb half an inch proximal to the interphalangeal joint.

Results.—The results of this operation have been uniformly good. The chief difficulty is to obtain the right degree of extension and flexion at the metacarpal joint, but even if this is not completely successful, the thumb is restored to a good position of abduction and a strong grasp becomes possible.

†7. *Tenodesis of the flexor longus tendon of the thumb (devised at Vellore).*

Indications.—A hyperflexed terminal joint of the thumb which has not been corrected by operation 6, but in which there is no joint damage.

Method.—The joint is forced into extension or slight hyper extension, if necessary, a skin graft may be used as in operation 2. The flexor

pollicis longus is then split into two from its insertion back for about an inch and one half of the tendon is detached from its insertion. This half is threaded into a drill hole into the neck of the proximal phalanx and fixed there with a stainless steel suture, while the terminal joint of the thumb is in full extension. This prevents the flexor longus from flexing the thumb beyond a chosen angle, but does not prevent it from controlling the thumb beyond this angle. The most useful and practical angle for pinch is about 180°.

Results.—This operation has not been used extensively, but has proved very satisfactory where it has been used.

8. *Interphalangeal arthrodesis of the thumb.*

Indications.—A hyper-flexed terminal joint of the thumb in which there is bony joint damage.

Method.—The thumb is forced into extension and the cartilage chiseled off until the two phalanges fit together with the joint in full extension. A fine Kirshner wire may be used to maintain the position.

Results.—Usually satisfactory providing immobilization is maintained sufficiently long post-operatively.

9. *'Z' plasty of thumb web.*

Indications.—For contraction of the thumb following long continued thumb paralysis. This contraction forms a block even to passive abduction of the thumb into the position of opposition and must be corrected before operation 6 can be performed for thumb reconstruction.

Method.—A 'Z' plasty of the thumb is made in which the first incision runs from the base of the thumb to the base of the index finger along the free edge of the thumb web. The second incision begins on the index-finger end of this incision and runs down the dorsum of the web at approximately 60° to the first incision. It is the same length as the first incision. The third incision begins at the thumb end of the first incision and runs down the thenar eminence at about 60° to the first incision and for the same instance. The two triangular flaps thus outlined are separated and the space between the first and the second metacarpals is opened up and all adhesions divided. The thumb origins of the first dorsal interosseus and the abductor pollicis muscles may require division. With the thumb placed in the abducted position the two flaps are transposed and the new 'Z' incision sutured.

Results.—Providing the thumb web has been not too severely contracted the results are good.

†10. *Sliding flap transfer (devised at Vellore).*

Indications.—Following long continued paralysis the thumb web becomes so severely contracted that no ordinary 'Z' plasty operation can restore enough stretch to the web to allow normal position of the thumb. The use of a sliding flap from the dorsum of the hand has, therefore, been developed.

Method.—The operation is like a 'Z' plasty, with an incision along the free edge of the web from the junction of the web with the thumb. The incision continues up on to the index finger, on to the dorsum distal to the metacarpo-phalangeal joint. It then turns sharply back at an angle

of 60° and sweeps right across the whole of the dorsum of the hand back to the wrist. The third incision begins at the junction of the web with the thumb and runs on to the palm round the thenar eminence at 60° to the first incision. The flaps outlined are raised, carefully preserving their blood supply and the thumb metacarpal is freed from the second metacarpal as in operation 9. When the thumb is pulled into abduction the large flap from the dorsum of the hand will be pulled across to cover the defect in the thumb web and the flap of skin from the dorsum of the index finger will lie across the web and turn down between the palm and thenar eminence. This leaves a large raw area on the dorsum of the hand. This raw area is covered with a full thickness skin graft from the trunk. A Kirshner wire is drilled through the thumb metacarpal across the web space and into the second metacarpal and left in that position for six weeks to keep these two bones apart during the healing of skin flaps.

Results.—Very satisfactory.

*11. *Wrist fusion and tendon transplantation for extensor paralysis.*

Indications.—When any extensor muscle is paralysed, commonly all the extensors are paralysed ; there is also usually complete ulnar loss. If it should happen that only one or two extensors are lost, other extensors should not be used for transplantation, they will probably become paralysed later. The following standard procedure may be used in all these cases :

First operation.—Wrist arthrodesis using a sliding graft from the radius and fixation with Kirshner wire.

Second operation.—Extensor transplant for the fingers and thumb bringing the flexor carpi radialis round the radial border of the wrist as a thumb extensor and the pronator radii teres or the palmaris longus round the ulnar side of the wrist to act as the extensor for the fingers.

Third operation.—A sublimis transplant for fingers and thumb (operation 1 and 6 referred to above).

Results.—This turns a completely useless hand into one sufficiently useful to co-operate well with the other hand in a manual trade.

12. *Arthrodesis of terminal joints of the fingers.*

Indications.—For acute irreversible flexion of the terminal joints of the fingers (especially index).

Method.—Two operations are possible : (i) Arthrodesis at about 20 degrees of flexion and (ii) fusion of the flexor profundus at the neck of the intermediate phalanx using stainless steel wire.

Results.—Not frequently performed, but fulfills its objects when it is performed.

13. *Ulnar nerve stripping and transplantation.*

Indication.—For acute recent paralysis of the ulnar nerve accompanied by ulnar nerve swelling, or may be used prophylactically to prevent ulnar nerve paralysis where such is anticipated.

Method.—The sheath of the ulnar nerve may be stripped from the area between the elbow and the upper end of the swelling. This is commonly four to five inches above the elbow. The sheath should not be removed entirely from the nerve, but should be stripped from end to end and left in continuity. This is in order to prevent accidental division of

the branch to the profundus or the branch to the carpi ulnaris which may lie in the sheath. It may also be of value to lift the nerve out of the bony groove behind the elbow and transpose it in front of the elbow. This can only be done if the muscular branches below the elbow can be stripped back sufficiently to allow the nerve to lie in this new position without tension.

Results.—There is no doubt that stripping will sometimes cause relief of pain and some relief of paralysis. The extent to which both of these procedures are of permanent value has not yet been fully proved.

14. *Median nerve stripping and carpal tunnel decompression.*

Indications.—For acute paralysis of the median nerve or early commencing paralysis or as a prophylactic against anticipated paralysis.

Method.—The roof of the carpal tunnel is divided and excised. The division should be made through the ulnar side of the carpal tunnel in order to avoid damage to the median nerve branches, such as the palmar cutaneous branch. The sheath of the median nerve may then be stripped from the wrist level back for about as far as the swelling continues, or for three to four inches. The roof of the carpal tunnel should not be resutured.

Results.—This operation is probably of value, but not enough cases have been followed up long enough for a definite opinion.

PREVENTION.

As one gets nearer to a complete understanding of the extent of the damage of nerves in leprosy and to an estimate of the extent to which one can overcome the results of nerve damage, the problem of prevention takes on a greater importance.

It has to be confessed that very little progress has been made in our understanding of the basic causes of nerve damage in leprosy since the last report. Some help has been received with regard to the planning of future investigations along these lines from a visit abroad. In particular, we wish to acknowledge the co-operation of Washington University at St. Louis and Professor Cowdry, in placing their electron-microscope and team of experts at our disposal in March this year. Some leprosy nerves were examined and a number of excellent electron-photo-micrographs of diseased nerves prepared. Further sections are being sent for a continuation of this study. This has been interesting, but it is too early to attempt to draw conclusions until one can be more sure of the interpretations of electron-microscopic findings in nerve pathology.

A programme of monkey-nerve experimentation has been started which Dr. Denney Brown of Boston helped to plan and it is hoped that experimental lesions in nerves may be produced which will help in the understanding of the lesions in leprosy.

4. **Leprosy inquiry under Dr. S. D. Desai at the Acworth Leprosy Home, Bombay.**

The work done can be grouped under two main heads :—

I. Examination and follow-up of new cases attended during the year 1952-53.

II. Examination and follow-up of old cases of previous years attended during 1952-53,

I. Examination and follow-up of new cases.

Table I gives the results of the clinical and bacteriological examination of some of the contacts of both the neural and lepromatous patients :—

TABLE I.

	Neural	Leproma
1. Number of patients whose contacts were examined	139	95
2. Number of contacts in the homes of patients ...	497	357
3. Number of contacts examined clinically and bacteriologically ...	243	175
(a) Proportion of contacts examined ...	48·89 per cent	49·01 per cent
4. Number of leprosy cases detected :		
(i) Adults ...	20*	24†
(ii) Children ...	14‡	31§
Total ...	34+	55±
(a) Proportion of leprosy cases detected to contacts in 3 above ...	14 per cent	31·43 per cent
(Cases with basic lesions excluded)		
5. Number of cases with basic lesions detected :		
(i) Adults ...	17	6
(ii) Children ...	37	29
Total ...	54+	35±
(a) Proportion of cases with basic lesions detected to contacts examined in 3 above ...	22·22 per cent	19·43 per cent
6. Number of contacts without lesions examined clinically and bacteriologically ...	155	85
(a) Proportion of contacts without lesions examined clinically and bacteriologically to contacts examined in 3 above ...	63·78 per cent	49·14 per cent
7. Number of contacts without lesions showing acid-fast bacilli in the skin :		
(i) Adults ...	56	39
(ii) Children ...	66	32
Totals ...	122	71
(a) Proportion of contacts without lesions showing acid-fast bacilli in skin to contacts without lesions in 6 above ...	78·77 per cent	83·54 per cent

*Of the 20 leprosy cases in adults, 5 were persistent lesions—early neural, 12 were anæsthetic and tuberculoid macules, 2 were moderately advanced neural and 1 was early lepromatous.

†Of the 14 leprosy cases in children, 3 were persistent lesions—early neural, 9 were anæsthetic and tuberculoid macules, 1 was early lepromatous and 1 was moderately advanced lepromatous.

‡Of the 24 leprosy cases in adults, 56 were persistent lesions—early neural, 12 were anæsthetic and tuberculoid macules, 4 were moderately advanced neural, 1 was early lepromatous and 1 was moderately advanced lepromatous.

§Of the 31 leprosy cases in children, 9 were persistent lesions—early neural, 20 were anæsthetic and tuberculoid macules and 2 were early lepromatous.

TABLE II.

Fifty-four cases with basic lesions— $\left\{ \begin{array}{l} \text{Bacillary positive} \\ \text{Lepromin positive} \end{array} \right.$

(Contacts of neural patients).

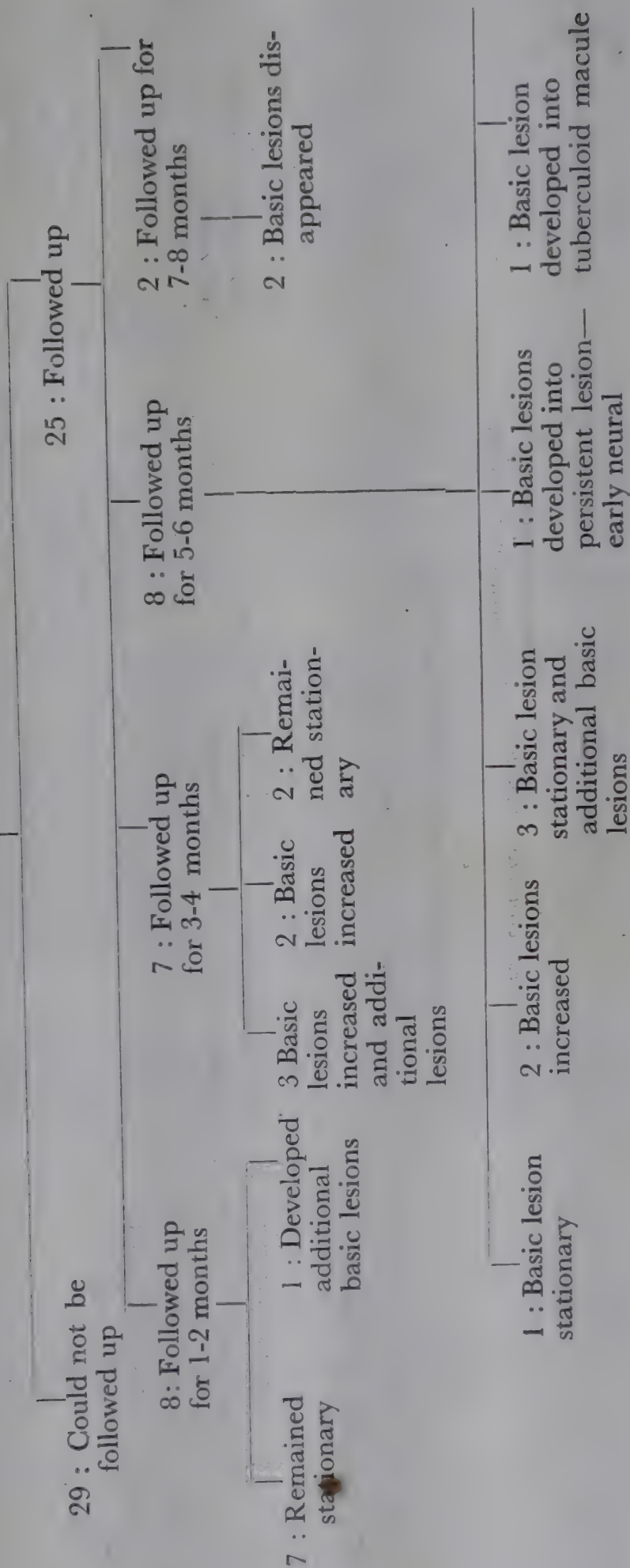


TABLE III.

Thirty-five cases with basic lesions — { *Bacillary positive*
Lepromin positive

(Contacts of lepromatous patients).

17: Could not be followed up			18: Followed up		
3: Followed up for 1-2 months			2: Followed up for 7-8 months		
2: Basic lesions stationary	1: Developed additional lesion		1: Basic lesion stationary	1: Basic lesion increased and additional lesions	
8: Followed up for 3-4 months			5: Followed up for 5-6 months		
1: Basic lesion stationary	2: Original lesions disappeared and new basic lesions developed		2: Basic lesions increased and additional lesions	1: Basic lesion stationary	
1: Basic lesion disappeared			2: Basic lesions increased and additional lesions		
1: Basic lesion stationary	2: Basic lesions increased		2: Additional basic lesions	2: Basic lesions increased and additional lesions	

Details of follow-up of cases with basic lesions mentioned in Table I (No. 5-i and ii).—‘Basic lesions’ is the term applied to the lesions commencing in positive contacts. These lesions were described by us as primary lesions (*Indian Journal of Child Health*, June 1952 and the Note of Work done by the Leprosy Inquiry at the Acworth Leprosy Home for 1951-52), as they were the first to appear and histology showed them to be the earliest lesions described so far. It was later found that such lesions developed after one similar lesion had already appeared in the same individual. Hence, the term ‘basic lesions’ is now applied to such lesions.

+The follow-up of 54 cases is given in Table II.

±The follow-up of 35 cases is given in Table III.

It will be seen that :—

- (1) The incidence of leprosy cases detected among the members of households of the neural and lepromatous patients is 14 and 31.43 per cent, respectively. All these had well-established leprosy lesions in early stages, but there was also a group of contacts with basic lesions. The incidence of such cases is 22.22 and 19.43 per cent in contacts of neural and lepromatous patients, respectively.

A study of the histological structure of such lesions has been undertaken by the Leprosy Inquiry under Dr. V. R. Khanolkar and a condensed description is given at the end of this note.

- (2) There was no significant difference in the incidence of contacts without lesions showing positive bacillary findings, viz. 78.77 and 83.54 per cent in contacts of neural and lepromatous patients, respectively. The proportion of contacts without lesions positive for bacilli (infected persons), may appear unduly large for a disease considered the least infectious of the infectious diseases; but on the follow-up of 68 such positive contacts, only 37, i.e. 54.41 per cent were found to develop leprosy.

Results of the lepromin test in relation to bacillary findings in contacts.—Two hundred and thirty-six contacts were examined bacteriologically and immunologically during 1952-53. The results are given in Table IV :—

TABLE IV.

(1) Number of leprosy cases studied	234
(2) Number of contacts living with patients	854
(3) Results of the lepromin reaction observed in contacts examined clinically and bacteriologically in				
(i) Adults	113
(ii) Children	123
Total	236

TABLE IV—(contd.)

		<i>Adults</i>	<i>Children</i>	<i>Total</i>
Infected persons	Bacillary positive Lepromin positive } ...	95	85	180
	Bacillary positive Lepromin negative } ...	Nil	12	12
	Bacillary negative Lepromin negative } ...	18	26	44
Uninfected persons	Bacillary negative Lepromin positive } ...	<i>Nil</i>	<i>Nil</i>	<i>Nil</i>
Totals		113	123	236

Note.—Lepromin positive :

Early reaction.—An erythema with œdema of 5 mm. and above round the site of injection occurring at the end of 24 hours.

Late reaction.—A hard nodule of 2 mm. in diameter and above at the end of three weeks. The significance of a nodule less than 2 mm. in diameter is being investigated.

II. Examination and follow-up of old cases of previous years.

In continuation of the ' Study of early lesions developing in contacts ', described in the report for the year 1951-52, it was possible to follow up 179 contacts examined during previous years. The condition noted from time to time in these 179 contacts is grouped and described as follows :—

- A : 70 cases followed up from the negative contact or uninfected stage (Bacillary negative and lepromin negative)—Table V.
- B : 68 cases followed up from the positive contact or infected stage (Bacillary positive and lepromin positive)—Table VI.
- C : 24 cases followed up from the positive contact or infected stage (Bacillary positive and lepromin negative)—Table VII.
- D : 15 cases followed up from the basic stage (Bacillary positive and lepromin positive)—Table VIII.
- E : 2 cases followed up from the persistent lesion—early neural stage (Bacillary positive and lepromin positive)—Table IX.

TABLE V.

Group A—*Seventy negative contacts*—{ Bacillary negative
Lepromin negative

(Followed up from six months to three years).

11 : Remained negative contacts	1 : Became bacil- lary + lepromin —	40 : Became bacil- lary + lepromin +	13 : Developed basic lesions bacillary + lepromin +	2 : Developed per- sistent lesions—early neural bacillary + lepromin +	3 : Developed tuber- culoid macules bacillary + lepromin +
	33 : Remained bacillary + lepromin +		6 : Developed basic lesions bacillary + lepromin +	1 : Developed persistent lesion—early neural bacillary + lepromin +	

TABLE VII.

Group C—24 positive contacts— $\left\{ \begin{array}{l} \text{Bacillary positive} \\ \text{Lepromin negative} \end{array} \right.$
(Children)

(Followed up from six months to three years).

10 : Remained bacillary + lepromin +		14 : bacillary + lepromin +	
4 : Remained bacillary + lepromin +	7 : Developed basic lesions bacillary + lepromin +	2 : Developed basic lesions bacillary + lepromin +	1 : Developed persistent lesions early neural bacillary + lepromin +
		which later deve- loped into persis- tent lesion —early neural bacillary + lepromin +	which later deve- loped into a tuberculoid macule bacillary + lepromin +

TABLE VIII.

Group D—15 cases with basic lesions— $\left\{ \begin{array}{l} \text{Bacillary positive} \\ \text{Lepromin positive} \end{array} \right.$

(Followed up from 6 months to 1½ years).

14 : Remained basic lesions bacillary + lepromin +	1 : Developed persistent lesion— early neural bacillary + lepromin +
12 : Remained basic lesions bacillary + lepromin +	2 : Developed additional basic lesions bacillary + lepromin +
	which later became tuberculoid macules bacillary + lepromin +

TABLE IX.

Group E— 2 cases with persistent lesions— { Bacillary positive
(Early neural.) { Lepromin positive

(Followed up for 1½ years).

1: Remained persistent lesion—
early neural
bacillary +
lepromin +

1: Developed additional basic
lesions
bacillary +
lepromin +

It will be seen from Tables V to IX that in some cases all the stages in the development of lesions from the negative contacts or the uninfected persons have been observed, i.e. the positive contacts or the infected persons, the basic lesion, the persistent lesion—early neural, the tuberculoid macule (or the anæsthetic macule). In some cases, however, one or more of the intervening stages have not been seen, as these cases could not be examined at regular and frequent short intervals.

The development of the early lepromatous lesions has, however, not yet been observed to arise from any of the stages described, i.e. the negative contacts, the positive contacts (bacillary positive and lepromin positive), the positive contacts (bacillary positive and lepromin negative), the basic lesion or the persistent lesion—early neural.

HISTOLOGY OF THE EARLY LESIONS.

Fifty-three biopsies were taken by the Leprosy Inquiry under Dr. V. R. Khanolkar, from the basic lesions. Thirty of them have already been studied and their report is given in the Note of the work done by that Inquiry (*see* page 138-140). A condensed description of the histological structure of such basic lesions is given below :—

Proliferation of histiocytes around blood vessels ; stray bacilli, intracellular or in the corium and in a few cases inside nerve twigs. Nerves normal in the majority of cases. In a few cases, however, commencing nerve damage (fraying) seen.

5. Leprosy inquiry under Dr. B. B. Gokhale at the B. J. Medical College and Sassoon Hospitals, Poona.

1. *Synopsis*.—(1) Micro-chemical methods for estimation of histamine in amounts of 0.1 to 1.0 microgramme per 10 ml., suitable for routine analysis of blood samples (normal content about 10 microgramme per 100 ml.) as well as of urine have been studied.

(i) A volumetric method for the determination of histaminase activity in biological media has been tried and is under consideration.

(ii) A more sensitive colorimetric method for estimation of histaminase activity by using 2-4 di-nitrofluorobenzene is also under consideration.

When these methods were applied to test the blood serum and blood of a patient suffering from toxæmia of pregnancy— a low histaminolytic activity in serum as well as high histamine content of the blood was observed.

2. *Micro-chemical methods for estimation of histamine.*—Determination of histamine with 2-4 di-nitrofluorobenzene according to the method of McIntyre, Roth and Shaw (1950).

(i) Purification of histamine to be determined is carried out by using n-butanol and cotton acid succinate at pH 11 to 12.

(ii) Coupling of histamine with 2-4 DNFB to form a histamine derivative.

(iii) Purification of the histamine derivative is worked out by passing it through cotton benzonate and cotton butyrate with the use of sodium carbonate buffer.

(iv) Histamine derivative is eluted from cotton-butyrate tube by means of hydrochloric acid, and further it is quantitatively estimated by means of its light absorption.

Histamine estimation in human blood (Lubschez, 1950).—After precipitating all proteins and separating them from whole blood, n-butanol is added to the clear supernatant solution. At pH 11 to 12 histamine is absorbed by N-butanol from the blood. Further amberlite IRC-50 (cation-exchange carboxylic-type resin) absorbs all the histamine that is present in n-butanol when shaken with it for a specific period. Histamine from the amberlite is eluted by normal hydrochloric acid and is heated with sodium nitrite for 30 minutes in a water-bath. Further, colour is developed in cold by diazotizing with p nitro-analine. The colour is extracted with methyl-isobutyl-ketone in order to concentrate and to stabilize it. The extract is treated with veronal buffer (pH 7·7) to free it from other interfering substances. It is estimated by means of light absorption.

3. *Determination of histaminase activity in biological fluids.*

(A) *Kepler-Adler.*—The method is based upon the incubation of a non-hæmolysed serum with known amount of histamine solution in weak phosphate buffer pH 7·2 in the presence of an excess of indigo di-sulphonate and oxygen. After 24 hours the control serum and the test serum containing an excess of indigo compound are titrated against 0·002 n KMNO_4 . The difference between the two readings (that of control serum and test serum) indicates the amount of hydrogen peroxide formed by the action of histaminase on histamine and thus indicates the activity of the enzyme.

(B) *Histaminolytic activity of serum—di-nitrofluorobenzene colorimetric method* (Dr. Dodge).—The basis of this method is the incubation of serum with a known amount of histamine followed by the measurement of remaining histamine by coupling it with 2-4 di-nitrofluorobenzene.

The rate of destruction of histamine at the specific concentration is limited only by the amount of histamine present. Therefore, the reaction is of the first order. The activity may, therefore, be expressed in terms of the length of time needed to destroy half the added histamine, this 'half-life' being inversely proportional to the activity.

4. (i) Experiments were carried out by employing the above-mentioned methods to determine histamine content in rabbit's blood and high concentration of about 20 microgrammes per 100 mil. of blood was noted.

(ii) Very low serum histaminolytic activity as well as high histamine content in blood of patient suffering from toxæmia was observed.

PLAGUE

1. Plague researches under the Director, Haffkine Institute, Bombay.

Nutritional requirements of P. pestis.—In the previous reports it has been stated that the influence of nuchar in enhancing the growth of *P. pestis* is neither due to (1) any amino-acid imbalance in the casein hydrolysate nor to (2) removal of any inhibitory factor from the casein hydrolysate. It was then considered that charcoal might act as a reducing agent in the medium. In the last year's report evidence has been adduced to show that growth of small inocula of *P. pestis* on nutrient agar is considerably enhanced by the addition of one per cent charcoal to the agar.

In order to determine the reducing effect of charcoal the following experiment was undertaken: Two isonitrogenous sets of casein hydrolysates, one charcoal-treated and the other without charcoal treatment were prepared from the same batch of casein, and inoculated with *P. pestis* (same inoculum 0.05 c.c. of 24-hour casein hydrolysate culture grown at room temperature). The potential of the medium was tested with the usual E_h (oxidation-reduction) indicators, such as benzyl viologen, neutral red, rosinduline, phenosafranine, Janus green, methylene blue, tolylene blue, o-cresol indeophenol and phenol blue. Changes in the oxidation-reduction potential are indicated by changes in the colours of these indicators. There were no observable differences in the colours produced in the two media during growth up to 15 days. This showed that charcoal by itself did not effect any changes in the redox potential of the media.

In order to see whether charcoal treatment in any way affects the reducing activities of *P. pestis* tetrazolium bromide (trade name Grodex, May and Baker product), which is unaffected by interfering substances, was used to test the reducing activities of *P. pestis*.

Tripheyl tetrazolium bromide is soluble in water and forms a colourless solution but in its reduced state (triphenyl formazon) the indicator assumes an intense red colour and is also insoluble in water. Triphenyl formazon is extracted by ether and since this indicator is unaffected by other reducing substances, any appreciable reduction of TTB to formazon may be ascribed to the reducing systems of bacteria during their growth and metabolic activities.

The colour intensities were compared in a Gallen Kramp photoelectric colorimeter using Ilford filter No. 622. A straight line was obtained for formazon concentrations ranging from 0 to 20 τ /ml. Triphenyl formazon for standardization was prepared from TTB by treatment with an equivalent quantity of ascorbic acid in presence of alkali.

0.5 c.c. of a one per cent solution of Grodex was added to 10 c.c. of two sets of tubes of casein hydrolysates, one treated with charcoal and the other not so treated. It was observed that the reduction of TTB to formazon in plague broth (treated with charcoal) was about 5 to 7 times that in casein hydrolysate not treated with charcoal, although the charcoal-treated broth gave only twice as much growth of *P. pestis* as the untreated broth. This evidence suggests that charcoal treatment of the broth considerably enhances the reducing systems of bacteria during their growth and metabolic activities.

At the suggestion of Sir Alexander Flemming that charcoal might probably remove certain inhibiting fatty acids which may be present in commercial casein, the influence of addition of graded amounts of oleic acid on the growth of *P. pestis* in casein hydrolysate was investigated. Five hundred mg. of oleic acid originally dissolved in ethyl alcohol was later on diluted with water so as to contain 0.5×10^{-5} to 0.5×10^{-7} of oleic acid in casein-hydrolysate broth. In the concentrations tested, oleic acid did not show any demonstrable effect on the growth of *P. pestis*.

Pulicidal value of pyrethrum.—Previously the knock-down residual effect of the powder formulation of pyrethrum extract alone was found to last for 21 days only. Last year experiments done with extracts of pyrethrum flowers mixed with a booster had shown that the powder formulation gave a fairly long residual effect till the time of observation, *viz.* six weeks.

Subsequently, 91 preliminary experiments have been done this year so far by spraying or dusting this insecticide in glass-jars, on bricks, plaster of Paris blocks, artificial rat-burrows and testing the same in the Laboratory, air-conditioned room and in glass-house. The test insects were a mixture of *Xenopsylla cheopis* and *X. astia* bred in this Laboratory. Twenty fleas were used in preliminary and 100 in final trials. These trials were done with powder, solution and emulsion formulations. These formulations were compounded by mixing either the extract with China clay or the crushed flowers with China clay or mixing the extracts with different solvents. The solution and emulsion formulations were used at 10 c.c. per sq. ft. and the powder formulation at 25 mg. per sq. ft. of space. The flowers from which the extract was prepared were procured from the Nilgiris, Assam and Jammu. The total pyrethrin content of these was 1.7 per cent, 1.5 per cent and 1.2 per cent, respectively.

Table I shows the immediate knock-down effect and the longest residual effect obtained by the three formulations in the Laboratory. It is seen that the powder formulation has the longest residual effect.

Table II shows some of the significant observations on the immediate as well as the longest residual effect obtained by powder formulations when mixed with a booster piperonyl butoxide. These have been compared with DDT, BHC, Chlordane, Dieldrin and Parathion in the same formulation. Pure pyrethrum-flower powder or extract without the booster does not give such a long residual effect. The booster piperonyl butoxide is seen in Table II to give a longer residual effect than sesame oil.

The above experiments were done at ordinary room temperature. In order to see if temperature and humidity would have any effect on the knock-down effect, the experimental jars were transferred to air-conditioned rooms and the knock-down effect observed for two weeks. Table III shows the knock-down effect first in the Laboratory at ordinary room temperature and then in the air-conditioned room at 26°C. Table IV shows the knock-down percentage for two consecutive weeks in the air-conditioned room. The knock down was better than in the room at ordinary temperature.

TABLE I.

Maximum residual effect obtained by some of the formulations.

Material used	Formulation concentration	Per cent knock down 1st day		Residual effect Number of days	Per cent knock down		Mode of application
		6 hours	24 hours		6 hours	24 hours	
Pyrethrum extract <i>plus</i> China clay 1:9	Powder 25 23 mg./sq. ft.	45	100	112	25	100	In glass-jars.
" " <i>plus</i> piperonyl butoxide ...	"	100	100	336	25	100	" " "
" " <i>plus</i> sesame oil ...	"	25	100	111	25	100	" " "
DDT ten per cent <i>plus</i> China clay 1:9	"	20	100	336	10	100	" " "
Piperonyl butoxide <i>plus</i> China clay 1:9	"	20	100	262	0	55	" " "
Pyrethrum-flower dust <i>plus</i> China clay 1:9	"	55	100	60	0	100	" " "
" " dust <i>plus</i> P.B.	"	45	100	66	0	100	" " "
Pyrethrum extract ...	Emulsion 10 c.c./sq. ft.	25	100	199	15	100	" " "
" " " piperonyl butoxide ...	"	15	95	48	0	30	On brick in jar
" " " " " "	"	45	100	233	30	100	In glass-jar.
" " " " " "	"	0	95	48	0	30	On brick in jar.
Pyrethrum extract ...	Solution 10 c.c./sq. ft.	100	100	279	5	35	In glass-jar.
" " " " " "	"	10	50	17	0	50	On brick in jar.
" " " piperonyl butoxide ...	"	100	100	279	15	100	In glass-jar.
" " " " " "	"	60	90	17	0	30	On brick in jar.
DDT (Hexamar) ...	"	100	100	279	55	100	In glass-jar.
" " " " " "	"	60	100	17	0	0	On brick in jar.

Residual effect seen in jars.
(Twenty fleas were used in each experiment. Only 15 minutes contact was allowed to the fleas and they were then kept for observation in a clean tube for 24 hours).

Name of the product used	Concentration	Method of application	Per cent knock down on 1st day		Residual effect per cent knock down			Remarks
			6 hours	24 hours	Number of days	6 hours	24 hours	
Powders								
Pyrethrum extract two per cent plus China clay 1 : 9	25 mg. per sq. ft.	Dusted in jars kept closed	100	100	222nd day	40	100	Discontinued
Pyrethrum extract two per cent plus 20 per cent piperonyl butoxide plus China clay 1 : 9	"	"	100	100	336th day	30	100	
" " "	"	"	100	100	"	25	100	
" " "	"	"	20	100	"	30	100	
DDT ten per cent in China clay	"	"	35	100	198th day	10	100	Discontinued
" " "								
Pyrethrum extract two per cent plus 20 per cent piperonyl butoxide plus China clay 1 : 9	"	"	75	100	"	60	100	"
Pyrethrum extract plus sesame oil plus China clay 1 : 9	"	"	25	100	111th day	0	95	
Pyrethrum plus sesame oil plus China clay	"	"	25	100	108th day	0	80	
DHC ten per cent plus China clay	"	"	85	100	194th day	100	100	"
Chlordane five per cent	"	"	30	100	306th day	25	100	
Dieldrin ten per cent	"	"	30	100	306th day	20	100	
Parathion 14 per cent	"	"	15	100	301st day	5	100	
Pyrethrum powder plus China clay 1 : 9 plus 20 per cent piperonyl butoxide	"	"	45	100	66th day	0	100	
Piperonyl butoxide plus China clay 1 : 9	"	"	20	100	262rd day	0	55	
China clay	"	"	0	0	39th day	0	10	Control

TABLE III.

Knock-down effect of pyrethrum formulations in the air-conditioned room as compared to that in ordinary room (B).

Material used	Concen- tration	Method of appo- intment	Number of days after the first experiment	Per cent knock down on 16-7-53 at 26°C. and 58 per cent rela- tive humidity		Number of days after the first experiment	Per cent knock down on 8-9-53 at 29°C. and 85 per cent rela- tive humidity (B)	
				6 hours	24 hours		6 hours	24 hours
Pyrethrum extract <i>plus</i> China clay 1 : 9	25 mg. per sq. ft.	Jar	57	45	95	112	25	100
Pyrethrum extract <i>plus</i> piperonyl butoxide 1 : 9	"	"	48	60	100	103	55	100
Pyrethrum <i>plus</i> sesame oil <i>plus</i> China clay 1 : 9	"	"	57	45	100	111	0	95
Piperonyl butoxide <i>plus</i> China Clay 1 : 9	"	"	207	65	100	262	0	55
DDT ten per cent <i>plus</i> China clay 1 : 9	"	"	279	75	100	334	30	100
Chlordane five per cent	"	"	250	55	100	305	25	100
Dieldrin one per cent	"	"	250	95	100	305	20	100
Parathion 14 per cent	"	"	245	55	100	300	5	100
Pyrethrum <i>plus</i> piperonyl butoxide emulsion	10 c.c.	"	258	10	40	313	30	100
Pyrethrum solution	"	"	224	60	90	279	5	35
Pyrethrum <i>plus</i> piperonyl butoxide solution	"	"	224	60	100	279	15	100
Hexamer solution (DDT)	"	"	224	85	100	279	55	100

TABLE IV.

The glass-jars were kept in the air-conditioned room (26°C. and 58 per cent relative humidity) 24 hours prior to the experiments. The fleas were exposed for 15 minutes and all the observations made in the same room. The temperature and humidity in this room was constant.

Material used	Per cent knock down 1st week :				Per cent knock down 2nd week :				Per cent knock down of the 2 weeks readings :		
									Mean		
	3 hours	6 hours	24 hours		3 hours	6 hours	24 hours		3 hours	6 hours	24 hours
Pyrethrum extract <i>plus</i> China clay 1:9	25	40	100		5	45	95		15	42	97
" " " piperonyl butoxide 1:9 ...	50	70	100		25	60	100		37	65	100
Pyrethrum <i>plus</i> sesame oil <i>plus</i> China clay 1:9	10	40	100		0	45	100		5	42	100
Piperonyl butoxide <i>plus</i> China clay 1:9	30	60	100		35	65	100		32	62	100
DDT ten per cent <i>plus</i> China clay 1:9	65	90	100		5	75	100		35	82	100
Chlordane five per cent	10	55	100		10	55	100		10	55	100
Dieldrin one per cent	20	75	100		85	95	100		52	85	100
Parathion 14 per cent	20	60	100		20	55	100		20	57	100
Pyrethrum <i>plus</i> piperonyl butoxide emulsion	15	30	100		0	10	40		7	20	70
Pyrethrum solution	0	60	100		30	60	90		15	60	95
Pyrethrum <i>plus</i> piperonyl butoxide solution	15	60	100		20	60	100		17	60	100
Hexamar solution (DDT)	25	75	100		15	85	100		20	80	100

Experiments done in the glass-house on the terrace have so far shown that the residual effect of the powder formulation of pyrethrum extract lasts only for sixteen days, while that mixed with piperonyl butoxide has continued for the last 29 days. These experiments, where the formulations are exposed to natural heat and light, are still continued.

It will be seen from the results in the above tables, that of the three formulations, powder formulation gives better residual effect when mixed with a booster. The booster piperonyl butoxide is better than sesame oil. The knock down is better in air-conditioned room and the residual effect is longest when tested in glass-jars not exposed to sun and light.

The cost of making the powder formulation comes to about Rs. 2 per lb. when extracted with methanol and other high-grade solvents and Re. 1 per lb. when done with kerosene oil.

2. Inquiry into the breeding habits of rat-fleas in relation to the epidemiology of plague under Dr. S. C. Seal at the All-India Institute of Hygiene & Public Health, Calcutta.

The purpose of this inquiry was to investigate into the bionomics of rat-fleas in Calcutta by a co-ordinated field and laboratory studies with a view to determine the seasonal, environmental and other conditions promoting or otherwise influencing the breeding of the insect and their relationship with the prevalence of plague in the city and with the endemicity of plague in general.

The whole life-cycle of each of the two rat-fleas, *X. cheopis* and *X. astia*, generally present in the city, was followed from the time of egg laying to the death of the adult in the Laboratory at all seasons of the year. The techniques developed for identifying live fleas and for rearing them up as pure strain and the results obtained during the first six months of this investigation were reported last year. A full year's study has since been completed. The results are summarized below :—

A. LABORATORY STUDIES.

1. Bionomics of rat-fleas—*X. cheopis* and *X. astia*.

(a) *Egg laying*.—It was interesting to note that neither *X. cheopis* nor *X. astia* developed fully mature eggs when fed on humans but they readily (68·7 per cent of *cheopis* and 73·1 per cent of *astia*) did so when fed on rats. The egg laying by *cheopis* was maximum during the cold months and spring, and minimum during the summer and rainy season ; the most favourable period for *astia* was autumn or early cold weather (October to December), the egg laying being minimum when the cold sets in. The average number of eggs also varied accordingly. This results was also corroborated by actual fertilization experiment. The efficiency of fertilization of freshly hatched *X. cheopis* were 25·0, 52·2, 41·6 and 61·5 per cent in the rainy season, autumn, winter and spring, respectively. The corresponding figures for *X. astia* were 45·4, 65·0, 48·0 and 20·0 per cent, respectively.

(b) *Larval development*.—Hatching of eggs were more facilitated during the cold weather than during the summer or rainy season for both the species. The average time taken for the hatching of eggs into larvæ was 4·3 days for *cheopis* and 3·9 days for *astia* with slight prolongation of the period during the cold weather. The rainy season was favourable for the larval development of both species, the average time taken to reach the pupal stage by *cheopis* and *astia* being 8·7 and 9·6 days, respectively, as against 10·6 and 14·4 days—the corresponding averages for the whole year. The development was slowest during the cold season, being 12·0 and 20·0 days, respectively.

(c) *Pupal cycle and adult hatching*.—The rainy season was also found favourable for quicker hatching of pupæ into adults of either species, being 10·6 days for *cheopis* and 9·4 days for *astia* as against the averages of 12·2 and 11·1 days for the whole period. The time taken was, however, longest, e.g., 18·0 and 17·3 days, respectively, during autumn (October to December).

It will be seen from above that the efficiency of development of the species of fleas varied in different seasons and at different stages of their life cycle. The sum total effect of these variations is given in Table I :—

TABLE I.

Developmental efficiency of X. cheopis and X. astia at different stages of their life-cycle and in different seasons as tested in this Laboratory.

Period	Species and number of eggs observed	Per cent larvæ hatched	Per cent of eggs into cocoon	Per cent eggs into adults	Average time taken in days	Average time taken to develop into an adult flea
Apr.-June, 1952	Xc 144	43·8	27·2	13·1	23·9	15·75
	Xa 161	51·5	22·3	22·9	25·2	15·5
July-Sept., 1952	Xc 150	40·0	32·6	28·0	23·5	20·3
	Xa 119	43·7	35·3	33·7	21·8	16·6
Oct.-Dec., 1952	Xa 61	80·4	31·1	31·1	37·8	24·5
	Xc 149	77·8	1·3	0·6	30·4	22·0
Jany.-Mar., 1953	Xc 176	73·9	52·2	46·0	30·1	16
	Xa 105	90·4	27·6	20·9	27·0	16·5
Apr.-June, 1953	Xc 230	17·8	4·3	4·3	29·8	...
	Xa 162	40·8	14·8	14·8	22·2	...
July-Aug., 1953	Xc 128	24·2	17·0	16·3	26·3	...
	Xa 271	40·2	32·1	30·2	24·2	...
Averages ...	Xc 889	41·6	26·1	21·6	28·4	...
	Xa 967	53·9	23·8	21·3	24·0	...

In the test-tube, on an average 21·6 per cent of *cheopis* eggs and 21·3 per cent of *astia* eggs ultimately became adults ; the former showed two waves of growth, the bigger one taking place in the cold season (Jan.-March) and the other in the rainy season (July-Oct.), while the latter has only one wave of rise in the rainy season (July-Sept.) with lowest developmental efficiency in the winter months. The average time taken by *cheopis* to become adults from eggs was 28·4 days as against 24·0 days by *astia*. Thus, the bionomical behaviour of *X. astia* partially accounts for its low-vector efficiency for the plague infection.

In the experimental cages, the entire life cycle of either species of flea was, however, completed in lesser time in properly set-up breeding cages than in the test-tubes. The results of these cage experiments differ from those obtained by Webster in Bombay but more or less resemble the findings of Hirst at Colombo and the Indian Plague Commission at Bombay.

Longevity of adult fleas.—*X. cheopis*, wild or newly hatched, starved or fed, generally lived longer than the similarly treated *X. astia*. Such longevity varied with seasons and was highest during the winter and lowest during the rainy season. Fleas bred in the laboratory lived longer than those caught from wild rats. The average periods of longevity of *X. cheopis* were as follows : Wild and starved 2·1 days, newly hatched and starved 4·5 days and newly hatched with one blood meal 6·7 days. The corresponding figures for *X. astia* were 1·8, 3·9 and 5·3 days, respectively. Individual *cheopis* having one blood meal could live for a maximum period of three weeks during the cold weather and spring as against 10 or 11 days for *astia*.

Sex distribution in different seasons.—The distribution of sex at different seasons was determined by sampling method in case of laboratory-bred fleas and wholesale examination in case of wild catches from wards 8 and 10 only. The results are given in Table II :—

TABLE II.

Sex distribution of the laboratory-bred and wild fleas by season.

Season	LABORATORY-BRED FLEAS				WILD CATCHES (WARDS 8 & 10)			
	<i>X. cheopis</i>		<i>X. astia</i>		<i>X. cheopis</i>		<i>X. astia</i>	
	M.	F.	M.	F.	M.	F.	M.	F.
Jan.-March ...	14	104	79	20	207	289	66	93
Apr.-June ...	179	367	86	113	377	280	163	255
July-Sept. ...	224	235	50	257	162	159	152	204
Oct.-Dec. ...	0	16	111	98	89	199

Table II will show that the female population of *X. cheopis* predominates in the winter months and early parts of spring and the males try to catch them up in the rainy season, whereas *X. astia* has more female population

during the second half of the year, which is partially replaced by males during the winter months. This behaviour, to a great extent, corresponds with their growth curves already discussed.

Feeding and biting habits.—*X. astia* bites and feeds better than *X. cheopis* but they bite rats more readily than man. The propensity is, however, less in the rainy season than in the winter months when *X. cheopis* takes feed as much as and even better than *X. astia*. The duration of feed is two to five minutes in man practically without any sensation, but a small red macule generally develops at the spot of the puncture within a few hours with or without itching. One-day old adults are less inclined to bite man than two days old, the proportions biting being 25·6 and 57·5 per cent, respectively.

Meteorological conditions during experimental period.—The temperature and humidity data under which the experiments were conducted at different seasons are given in Table III. The readings were taken at 10 a.m. and 4 p.m. daily on week days.

TABLE III.

Season		Maximum temperature	Minimum temperature	Dry-bulb temperature	Wet-bulb temperature	Relative humidity, per cent	Saturation deficiency
Jan.-March	...	81·3	77·9	71·9	69·2	86·8	0·105
Apr.-June	...	94·7	86·5	86·4	80·8	78·0	0·259
July-Sept.	...	89·4	85·8	80·4	79·5	96·0	0·031
Oct.-Dec.	...	84·1	79·2	73·1	72·4	96·2	0·017

Transmission experiment with laboratory bred-fleas.—So far two clear-cut transmission experiments, one with *X. astia* and another with *X. cheopis*, have been performed during August and September. Briefly, no transmission of plague infection could be effected through *X. astia* but it was readily successful with *X. cheopis* which could infect the third series of mice in succession within ten days. Further work is proceeding.

B. FIELD STUDIES.

Only two wards in the city, Nos. 8 and 10, were chosen for carrying out simultaneous field investigation. In ward 8, plague was persisting since its reappearance in 1948, while ward 10, though initially slightly involved, remained free from plague infection for the last four years. The former has been designated as 'endemic' and the latter as 'non-endemic' for the purpose of comparison.

Rodent densities and flea indices.—The relative distributions of the various species of rats in the two wards for the year 1952-53 have been compared in Table IV with those of the entire city at different times, and their densities at different seasons are given in Table V :—

TABLE IV.

Percentage distribution of different species of rats in the whole city and in wards 8 and 10.

Author and year	City of ward	<i>R. rattus</i>	<i>R. norvegicus</i>	<i>G. varius</i> (<i>Bandicota bengalensis</i>)	Other rodents
Hossack (1906)	Whole city	14·0	26·0	60·0	...
Rao (1936) ...	" "	13·5	22·0	27·3	37·2
Present study					
(1948-52)	" "	13·3	9·0	75·8	1·7
(1952-53)	Ward 8	28·6	13·1	58·3	...
(1952-53)	Ward 10	16·8	8·6	74·3	...

TABLE V.

Rodent densities in wards 8 and 10 by species and season.

Seasons	RODENT DENSITIES WARD 8 (ENDEMIC)				RODENT DENSITIES WARD 10 (NON-ENDEMIC)			
	Total	<i>R. rattus</i>	<i>R. nerved.</i>	<i>B. bengalensis</i>	Total	<i>R. rattus</i>	<i>R. nerved.</i>	<i>B. bengalensis</i>
Jan.-March ...	22·6	7·1	1·4	13·3	23·2	8·6	0·7	13·9
Apr.-June ...	42·5	18·1	6·1	18·3	10·2	0·0	3·4	6·8
July-Sept. ...	21·8	4·1	5·3	12·4	10·9	2·7	2·1	6·1
Oct.-Dec. ...	30·7	5·4	2·7	22·6	32·1	2·6	2·3	27·2
Whole year ...	29·8	8·5	3·3	18·0	22·5	3·6	1·9	15·9

The data given in Tables IV and V show that ward 8 is placed in a more favourable position than ward 10 in respect of both distribution and densities of *R. rattus* and *R. norvegicus*, to facilitate propagation of plague infection. There is also an increase in their densities during the winter and spring months in ward 8 and rather a decrease in *R. rattus* densities in ward 10.

The total and specific flea indices in wards 8 and 10 at different seasons are given in Table VI :—

TABLE VI.

Total and specific flea indices in wards 8 and 10 by seasons and species of rats.

Seasons	Ward 8			Ward 10			CHEOPIS-INFESTATION INDICES					
	<i>Cheopis</i> index	<i>Astia</i> index	Total index	<i>Cheopis</i> index	<i>Astia</i> index	Total index	Ward 8			Ward 10		
							<i>R. rattus</i>	<i>R. norvegicus</i>	<i>R. bengalensis</i>	<i>R. rattus</i>	<i>R. norvegicus</i>	<i>R. bengalensis</i>
Jan.-March ...	4.7	1.6	6.3	2.2	0.4	2.6	3.4	8.0	4.2	4.3	5.5	0.7
Apr.-June ...	2.4	0.9	3.3	1.5	4.8	6.3	2.6	6.3	0.5	0.0	3.7	0.3
July-Sept. ...	1.4	0.9	2.3	2.1	0.6	2.7	4.3	1.2	0.5	2.7	2.6	0.2
Oct.-Dec. ...	0.8	1.0	1.8	0.4	0.7	1.1	2.0	3.5	0.2	2.2	1.1	0.1
Whole year ...	2.0	1.0	3.0	1.1	0.8	1.9	2.8	4.1	0.9	3.3	2.3	0.3

Both total and *cheopis* indices were comparatively higher in ward 8 than in ward 10, particularly during the winter and spring months. The *cheopis* infestation was highest in *R. norvegicus* during the winter months in both the wards and it also remained high in all seasons except the rainy seasons in ward 8 and autumn in ward 10. The percentage of infested rats was 54.7 in ward 8 as against 33.3 in ward 10. On the whole *R. norvegicus* was found more generally infested than *R. rattus*, *B. bengalensis* having the lowest infestation rates. The proportion of *cheopis* to *astia* was 6.5 : 3.5 in ward 8 and 5.7 : 4.3 in ward 10. Thus, these findings are also more favourable in ward 8 for the spread of plague infection than in ward 10.

Examination of rat-burrows.—Altogether 180 burrows were examined for free fleas and for temperature and humidity. The percentage of samples showing fleas were 7.4, 18.5, 21.9, 6.9 and 0.0 in the months of April, May, June, July and August, respectively (average—10.5 per cent). Nearly all the burrows except three belonged to ward 8, ward 10 having very few open and approachable rat-holes. In August, many of them had water collection near the mouth. In all but eight instances the rat-holes were found in connection with tiled huts or tin-sheds. Out of 19 positive samples 7 had *X. cheopis* and 12 *X. astia*. In 16 instances larvæ were also recovered from which adults hatched out in six instances and the rest had already died. The difference between the outside and inside maximum and minimum temperatures were as follows: May—maximum 3°, minimum 7.9°; June—maximum 5.5°, minimum 2.5°; July—maximum 4.4°, minimum 3.0°; August—maximum 10.8°, minimum 3.2°. The humidities inside the burrows were always between 95 to 100 per cent.

Examination of fleas for plague infection.—This work was carried out in collaboration with the staff of the Plague Control Laboratory under the Government of West Bengal who collected the fleas. Only fleas collected from wards 8 and 10 were subjected to this examination. From May to August 450 *X. cheopis* (ward 8—104♂, 201♀; ward 10—45♂, 100♀) and 1077 *X. astia* (ward 8—118♂, 220♀; ward 10—243♂, 496♀) were examined by culturing on blood agar as well as by mice inoculation in 149 instances. No serologically typical *P. pestis* has so far been isolated.

CLINICAL RESEARCH

1. Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

SYNOPSIS.

(a) The clinical work on the estimation of vitamins B_1 , B_2 and B_{12} on administration of a test dose of estrogens and the level of estrogens after the administration of vitamins B_1 and B_2 on a selected number of hospitalized oral cancer patients is in full swing. So far fourteen cases have been completed. It is found that there is a deficiency of the vitamins B_1 , B_2 and B_{12} in oral cancer patients (Table I) and administration of vitamins increases the excretion of the estrogens (Table II). In six cases, it has been observed that excretion of vitamin B_1 (after administration of estrogens) goes down below the normal level (Table II). The decrease in the excretion of vitamins may be due to their retention in the body, thus supplying the extra vitamins needed for improving the cancerous lesions. It is interesting to note that there is a definite fall in the vitamin B_{12} excretion (on administration of estrogens) in all oral cancer cases. The studies on cancer patients have been continued.

(b) Estimations on adult healthy women for establishing normal levels have been undertaken. So far estimation of 17-ketosteroids, estrogens and pregnanediol in twelve healthy women with normal menstrual cycle have been carried out. It would be necessary to collect data from a larger group for establishing normal excretion levels of the hormones in persons of the given age-group.

(c) Partial adrenalectomy of the five-year old girl with masculine characteristics, referred to in the last year's report, was performed at the Tata Memorial Hospital. Post-operative clinical investigations have shown reduction in the pigmentation and in the excretion of 17-ketosteroids.

PROGRESS REPORT.

A study of the effects of administration of estrogenic hormones on the excretion of vitamins B_1 and B_2 and the level of estrogens after the administration of vitamins B_1 and B_2 in oral cancer cases, leukoplakia cases and normal persons has been undertaken.

So far fourteen oral cancer cases have been studied. Every patient is hospitalized for eight days, and 24 hours urine is collected every day under toluene. Vitamins (B_1 10.0 mg. ; B_2 12.0 mg. and nicotinic acid 100 mg.) are administered on the third and fourth day. No administration is done during the subsequent days. Then on the seventh and eighth day patients are treated with hormones (0.5 mg. estradiol tablets, oral, 3 mg. estradiol benzoate injection and 0.5 estradiol tablets, oral, 2 mg. estradiol benzoate injection, respectively).

The samples are then worked out as follows :—

First and second : Estimation of estrogens and vitamins to know the original levels.

Third and fourth : Estimation of estrogens to know the effect of vitamins on hormones.

Fifth and sixth : Estimation of estrogens and vitamins to see whether the levels have come to normal.

Seventh and eighth : Estimation of vitamins to know the effect of estrogens.

In addition to the estimation of vitamin B_{12} in urine the level in blood of B_{12} is also found as outlined below :—

First day : Normal level of B_{12} .

Fourth day : Blood taken $3\frac{1}{2}$ hours after the administration of vitamins to study the effect of vitamins B_1 and B_2 on B_{12} level.

Eighth day : Blood taken $3\frac{1}{2}$ hours after the administration of estrogens to study the effect of estrogens on vitamin B_{12} contents.

Deficiency of all these vitamins is observed in oral cancer patients (Table I) :—

TABLE I.
Initial levels of vitamins in 12 oral cancer cases.

Patient number	Thiamine	Riboflavin	Vitamin B_{12}
	Normal : 150-300 μ g./24 hours	Normal : 500-800 μ g./24 hours	Normal : 85-100 m. μ g./24 hours
1	91-93	374-403	27-29
2	23-25	29- 59	30-34
3	93-111	41- 46	22-23
4	25-26	117-128	31-31
5	33-44	143-186	55-55
6	15-17	300-306	21-21
7	56-62	139-142	38-38
8	29-31	60- 82	20-21
9	25-28	37- 53	24-24
10	23-28	32- 47	14-16
11	76-77	15- 52	26-27
12	50-56	38- 81	21-25

The following is a sample table showing the effects of estrogenic hormones on the metabolism of vitamin B_{12} and *vice versa* in oral cancer cases.

TABLE II.

Showing the effects of the treatment of estrogenic hormones on the metabolism of vitamins—B₁, B₂, B₁₂—and vice versa in oral cancer cases.

Sample	Treatment	Creatinine (g./24 hours)	Vitamin B (τ /24 hours)	Vitamin B ₂ (τ /24 hours)	Vitamin B ₁₂		Estrogens (τ /24 hours)
					In urine (m τ /24 hours)	In blood (m γ /24 hours)	
1	Nil	1.56	76.4	52.7	27.30		31.2
2	Nil (after 48 hours	1.12	77.2	17.2	26.94	1.08	53.8
3	vitamins)						
4	Vitamins	1.48	29.82	...	125.6
5	Nil	1.42	30.96	...	104.4
6	Nil	1.38	80.8	478.4	27.70	1.08	62.3
	Nil (after 144 hours Estro- gens)	1.41	92.2	256.0	25.60	...	44.8
7							
8	Estrogens	1.11	36.4	165.7	13.16
	Nil	0.96	18.0	100.9	5.25	0.58	...

(a) It may be pertinent to point out here that the methods for the determination of estrogens and vitamins are laborious and time consuming. A period of 15 days is necessary for obtaining complete data in each case covering 41 estimations. This is sufficient to indicate the time and the labour involved in the study of 14 cases (574 estimations) reported above. The work on this aspect of the problem has now advanced to a stage that it would be possible to summarize the entire data and send it for publication.

(b) A reference has been made in the Summary of the Report about this work. In addition nine normal males have been studied as regards the excretion of estrogens and 17-ketosteroids.

(c) Partial adrenalectomy was performed in the case of the girl with masculine development referred to in the last year's report.

There is a reduction in the pigmentation and hirsutism. The voice which was coarse and of lower pitch has also remarkably changed to a higher pitch. There is, however, no considerable reduction in the size of the clitoris or muscular development. The rickety appearance of the girl has also not improved. There is a definite fall in the excretion of 17-ketosteroids after operation. (6.45 mg./24 hours pre-operative to 3.85 mg./24 hours post-operative). Investigations of the case will be published shortly.

2. Neuropathological unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

1. *Studies on the sensory mechanism of the skin.*—The study of cutaneous innervation and sensation in leprosy was continued to completion. On

reviewing the entire material and correlating the clinical findings with the histological observations a few facts of general physiologic significance have emerged.

It has been noted that out of the total of 232 'whole mounts', there are 121 that were prepared from skin with *normal* sensibilities for tactile, thermal and pain stimuli. Of these 121, 76 were obtained from healthy-looking areas of skin (the control biopsies), and 45 were derived from early lesions of leprosy (42 lepromatous and 3 neural macules). Only two patterns of nerve formations have been consistently observed in all these vitally stained biopsies: the nerves either form circlets and baskets around the hair-root and follicle, constituting the 'trichoneural apparatus', or they dichotomise at different levels to form 'nerve-nets' made up of many fibres or a single fibre. The former represents the mechanism with the greatest tactile acuity; the latter subserves pain and probably heat and cold. In any case, only 6 of these 121 preparations revealed any form of organized nerve-endings. This implies that for the greater part of the body skin (the hairy skin), end-organs are not essential for the reception of cutaneous sensations. This conclusion gains corroboration from the recent report of Sinclair, Weddell and Zander (1952) on the histo-clinical correlations of the human pinna, and refutes the hitherto-held notion of specific end-formations rigidly subserving individual modalities of cutaneous sensation.

Further, clinical examination of the 156 lesions of this series (87 lepromatous and 69 neural) revealed loss or impairment only of pain, or of heat, or of both in 62 (37 lepromatous and 25 neural). Fifty-one of these (26 lepromatous and 25 neural) manifested a concomitant deficit of thermal and pain sensibilities. This observation, coupled with the absence of specialized thermal end-organs, suggests the possibility that perception of heat and cold is mediated by intra-dermal nerves similar, if not identical, to those that subserve pain, namely the finer and more superficially situated myelinated and non-myelinated fibres that compose the nerve-nets. This inference of a common neural mechanism for these two groups of cutaneous sensation finds support from electrophysiologic findings that the delta-group of fine medullated fibres (Bishop, 1946) and the C-group of finer non-medullated fibres (Lewis and Pochin, 1937) both individually subserve pain, heat and cold.

In the light of these findings it is felt that a modification of current concepts regarding the structure and function of sensory mechanisms of the skin is indicated.

2. *Effect of radiation on cutaneous nerves.*—An experimental study of pathological changes in cutaneous nerves following radiation is being undertaken with the technique of vital staining by methylene blue. Young rabbits are employed and skin of the ear has been selected for the study, since it can be easily irradiated, avoids any of the general effects of radiation and lends itself to easy biopsies.

Six rabbits have so far been used. Roentgen radiation is administered by contact, through a 40 KV machine, employing a single 1 mm. aluminium filter and a port 1" in diameter. The dorsal skin of the ear is irradiated; employing doses varying from 50 r to 11,700 r. Forty-three biopsies have so far been made at intervals varying from 1 day to 70 days after radiation. A few biopsies from un-irradiated skin have been made

for control. Either intra-vital or supra-vital staining with fresh 0.02 per cent methylene-blue solution is employed ; unlike human skin, the nerves of rabbits skin stain equally well with either method.

Peripheral nervous tissues is known to be resistant to any form of ionizing radiation (Warren, 1943) and rabbits' skin is reported to be much more resistant to radiation than human skin (Christensen *et al.*, 1952). These observations have been corroborated by the present study, for even with the high doses employed, no significant disintegration of the cutaneous nerves has been observed so far. An appreciable number of nerves, both small and large, appear sheathed with doses above 5,000 τ ; this may be an early indication of degeneration, since normal skins have not revealed an equivalent degree of sheathing.

Portions of ten biopsies have, in addition, been stained by the silver method ; the general appearance of nerves in these serial sections has been comparable to that observed in vitally stained whole mounts. Parts of ten other biopsies have been stained for cell studies ; inflammatory reaction has been conspicuous by its absence.

3. *Experimental lathyrism.*—Supplies of *L. sativus* obtained from the Nutrition Officer, Bihar, were repeatedly put through coarse sieves to remove the seeds of other species of lathyrus (viz. *L. aphaca* and *L. sphericus*, both of which are smaller than the former and are the only two other species present in India) and extraneous material. The final product so obtained was an almost pure sample of *L. sativus*. The seeds were then ground into coarse powder to be incorporated in the diets. Two groups of experiments are in progress : (A) on albino rats, and (B) on monkeys:—

(A) Young Wistar rats of a pure strain maintained in these Laboratories are employed. Following weaning the animals are separated and maintained for one to two weeks on stock laboratory diet. The experimental diets are constituted of varying proportions of the lathyrus powder with certain ether essential ingredients as given in Table I :—

TABLE I.

Summary of the experimental and control groups with their diets.

Diet		Experimental group number					
		a. (6)	b. (4)	c. (6)	d. (6)	e. (4)	f. (4)
Parts by weights	<i>L. sativus</i> powder	460	900	100
	Whole-milk powder	45	33	...	45	33	...
	Wheat flour	150	600	...
	Rice flour	310	300	...
	Cod-liver oil	10	18	...	10	18	...
	Sweet oil	40	18	...	40	18	...
	Liver extract	4	4	...	4	4	...
	NaCl	2.5	2.5	...	2.5	2.5	...
	CaCO ₃	2.5	2.5	...	2.5	2.5	...

Figures in brackets indicate number of animals in each group. a, b and c are experimental groups. d, e and f are control groups.

Observations.—Diets are fed *ad libitum*. Control group (e) was maintained mainly to serve as a check upon the effects of reduction in the other constituents of the diet necessitated by inclusion of *L. sativus* at a very high

level. Control group (f) (starvation) was maintained to rule out the effects of starvation in the lathyrus-fed animals. Experimental groups (a) and (b) and control groups (d) and (e) have been under observation for about four months. Weights are being recorded every few days and the animals regularly examined, particular attention being paid to their gait, fur, and general condition. All the animals of these four groups have been steadily gaining weight and have so far exhibited no ill effects, except for some roughening of the fur in the experimental rats on 92 per cent lathyrus (group b).

Experimental group (c) is under observation for the last three weeks. The rats appear pot-bellied and weak and have rough fur. Compared to the other two experimental groups, any gain in weight in these six animals is negligible. None of the animals of the above six groups have been autopsied as yet, as it is proposed to observe the effects of the diets for at least six months before sacrifice for histologic study.

B. Observations on monkeys have only been possible for the last $1\frac{1}{2}$ months owing to certain unavoidable difficulties in arrangements for housing these animals.

Four young *rhesus* monkeys accommodated in separate cages have been employed initially. The whole grain, either germinated or boiled in water, was found to be consistently unacceptable to these animals. *Chapatties* incorporating 80 per cent lathyrus powder, 12 per cent wheat flour and sweet oil, jaggery and salt to make up 100 per cent were then tried. Weights were taken of food placed in the cages every day and the balance left out the next morning. It was found that the consumption of these *chapatties* was erratic—the average daily intake per animal being about 50 g. to 100 g. only. Information subsequently received from the Nutrition Research Laboratories, Coonoor, indicates experience of similar difficulties in feeding a high content of lathyrus in the diet for monkeys. It has, therefore, been decided to reduce the percentage of lathyrus in the diet (keeping the form of administration the same) and gradually increase the lathyrus content by five per cent every few days to reach the maximum of about 80 to 90 per cent.

4. *Pathological examination of human brains in cases of malnutrition.*—Among other sources, material for this study is being obtained from autopsies performed on unclaimed bodies at the N.M. Mental Hospital, Thana. While whatever neuropathological material available from this institution is being acquired by this Unit, only such cases are finally included for this study as have overt manifestations of undernutrition. After a detailed gross examination and description, the brains are sectioned according to a uniform plan and a large number of blocks representative of all the important areas and levels of the brain embedded in celloidin and in paraffin. Stains for cellular details and fibre tracts are applied routinely.

5. One of the objects in establishing this unit was that it would serve as a reference centre for neuropathology. It is gratifying to note that a number of specimens are being referred to this unit for opinion. This includes neurosurgical biopsies, brains from routine autopsies and muscle biopsies from suspected cases of muscular dystrophy or neural muscular atrophy. All this material is being carefully studied and documented and reports forwarded.

3. Inquiry on the rôle of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao, at the Haffkine Institute, Bombay.

EXPERIMENTAL HEPATIC NECROSIS.

(a) *Production of hepatic necrosis in experimental animals by feeding hydrogen-peroxide-treated yeast as source of protein.*—It has already been reported that massive hepatic necrosis in experimental animals fed on yeast-necrogenic diets, has not been produced regularly in this Laboratory. It was considered that the presence of small amounts of methionine and cystine in the yeast used for experiments may be responsible for not obtaining uniform results. In order to eliminate this factor, a fresh experiment was planned in which the control group of eight animals received 8 g. of the yeast-necrogenic diet *plus* vitamin E (1 mg./rat/day), whereas the experimental group of 28 animals received the same quantity of the diet containing hydrogen-peroxide-treated yeast. The animals were on this diet for over a period of 60 days, yet none of the animals which died or sacrificed showed any signs of hepatic necrosis, both macroscopically and microscopically.

(b) *Effect of different levels of dietary yeast in the production of hepatic necrosis.*—It has previously been reported and also as shown in the preceeding experiments that in this Laboratory it has not been possible to produce hepatic necrosis in rats either by dietary deficiency of vitamin E, S-amino acids or simultaneous deficiencies of both. Recently, McClean and Pest (1952) have suggested that experimental hepatic necrosis can be produced in rats only at an optimum level of yeast, both higher and lower levels of yeast disavouring the production of the hepatic lesions. In order to ascertain whether there is any optimum level of dietary yeast which produced necrosis in the livers of experimental animals preliminary experiments were carried out as described below :—

Thirty-six young male albino rats of the Haffkine inbred strain were divided equally into six groups with respect to litter mates, weights, etc. and fed 'yeast-necrogenic diet' with yeast levels varying from 20 to 60 per cent. The rest of the diet was similar to that used in our previous experiments except that alcohol and ether extracted Brewer's yeast (Squibb's) was used as the source of yeast instead of dry yeast of Burgoyne & Co. and instead of arachis oil at a level of 0.5 c.c./rat/day, lard of Genuine Pork Products (Central Dairy Farm, Aligarh, U.P.) was substituted at the same level. The animals were fed this diet up to a period of 120 days. After 60 days on the diet, they were sacrificed at regular intervals and the liver studied histologically.

Results.—The liver sections of animals fed 60 per cent yeast showed normal lobular pattern; the parenchymal cells showed normal histological appearances. Occasionally, there were traces of fatty infiltration of the parenchymal cells. When the yeast level was at 50 and 40 per cent the liver sections showed similarly occasional fatty change in the parenchymal cells and vacuolation of the cytoplasm. At 30, 25 and 20 per cent yeast levels, the liver sections showed severe massive hæmorrhagic necrosis of the parenchymal cells. The sinusoids were dilated and engorged with blood. In severe cases, only very small patchy islands of parenchymal cells were seen around the portal tracts.

As these experiments are only of a preliminary nature, further work is necessary to confirm these results and to determine the critical level of yeast at which this massive hæmorrhagic necrosis can be produced regularly.

AMINO-ACID COMPOSITION OF HUMAN MILK.

'Kwashiorkor' and 'infantile biliary cirrhosis' are usually prevalent in children during the weaning period; i.e. when the breast-fed baby is abruptly transferred to predominantly starchy foods, such as 'cassava' or rice diets. Recently, Bigwood (1952) has stressed the fact that this might be due to a diminution in the sulphur supply to the body in a diet formed predominantly of carbohydrates. It has been widely recognized that the building of tissue proteins (particularly those of the skin and hair) involves a high demand for sulphur; the amino-acid methionine stands at the centre of the metabolic traffic between choline, vitamin B₁₂, cystine and creatinine. With a view to unravelling the deficiencies in children's diets, which are primarily responsible for liver diseases, it has been suggested by various workers that a complete amino-acid analysis of the milk of mothers, obtained from various economic and social strata of the society will yield reliable data regarding the position of particular amino acids in infant nutrition.

The amino acids were determined qualitatively by the paper-chromatographic technique (Block *et al.*, 1952) in about 35 samples of human colostrum and transitional milk secreted two to ten days *post partum*. The samples were collected from normal as well as under-nourished mothers in order to compare the pattern of amino acids in the milk proteins, particularly the presence of the sulphur-amino acids, methionine and cystine.

Results.—On examination of the chromatograms of the acid hydrolysate of the milk samples, ten spots were observed in the case of normal milk, while only six were found comparable with the standard in the case of samples of milk from under-nourished mothers. Tryptophan and cystine were absent in all the samples, probably due to their destruction during acid hydrolysis. Threonine, methionine, arginine and phenylalanine spots were not seen in the under-nourished mothers' milk samples. These observations require further study and so it is considered necessary to analyse more samples of milk from both the groups.

INFLUENCE OF FERMENTED FOODSTUFFS ON FATTY INFILTRATION OF THE LIVER.

Liver disease and its prevention are worldwide problems, especially over the tropical belt of the world, and there is a wide consensus of opinion that liver disease is found concomitantly with general malnutrition—a low intake of dietary protein associated with proportionately larger intakes of carbohydrate calories. At the same time, the incidence of liver disease is not so high in this country as one would expect from a perusal of our dietaries. It is well known that the people who take low-protein high-carbohydrate diets generally take numerous foodstuffs and beverages which undergo fermentation at some stage or other in their preparation, and it has been widely speculated that micro-organisms might contribute appreciably to a restricted dietary. Fermented foodstuffs may also lessen the

strain put on the digestive enzymes of the body, by dismutating the macromolecules of the diet to more assimilable forms ; thus, improving the efficiency of utilization of foods in digestive disturbances as found, for example, in Kwashiorkor. Recently, György (1952) has adduced evidence to show that 'fermentation of food may increase under special circumstances the biological value even of a low-protein diet'. In India people are used to taking fermented foods in one way or other. Dahi (fermented milk) is *sine qua non* of most Indian meals. Other items are *iddalee*, *dosai*, leavened bread, *dhokla*, etc.

Experimental studies on the effects of supplementation of some fermented foodstuffs on fatty livers produced by high-fat low-protein diets are in progress.

4. Inquiry into typhus in Bengal under Dr. K. V. Krishnan at the All-India Institute of Hygiene & Public Health, Calcutta.

The work done during this year was in part a continuation of that reported last year, i.e. in experiments to infect a brood of mites (*T. deliensis*) obtained from an area from where no Scrub typhus had ever been reported.

Up to the date of report, 12 linear generations of mites have been fed on infected rodents with negative results. The first seven generations were fed on Laboratory-infected mice, the eighth generation on artificially infected rats (*R. rattus* and *B. bengalensis*) bred in the Laboratory. The ninth and tenth generations were fed on naturally infected wild rats, and the eleventh and twelfth generations on artificially infected white mice.

The inoculation, intraperitoneally into mice, of as many as 1,000 crushed larvæ from each generation, failed to show any evidence of infection. The mites were bred in tubes in which fungi of various sorts were encouraged to grow and controlled by a species of earthworms when they grew too luxuriant and threatened to overwhelm the nymphs and adults.

Sections of the ears of proved positive rats were made and stained to determine the presence of a localized rickettsial infection in the reticular layer of the skin. No infections were detected.

That rickettsiæ were present in adequate numbers in the peripheral blood of infected mice was proved by showing that four drops of blood was sufficient to cause infection in a second mouse when injected intraperitoneally.

The fact that 12 generations of mites fed on infected rodents failed to take the infection led to the idea that there may be physiologically different races of *T. deliensis*.

Before such an assumption could be accepted it is necessary to point out that systematists are not all agreed whether *T. deliensis* is a specific species or only a variant of *T. akamushi*. Several populations of *T. deliensis* have been studied and their standard data treated statistically. It was concluded that this is a very variable species, the various populations showing differences in the standard data.

It was, therefore, decided to raise a colony of infected mites and for this purpose rats were collected from typhus infected areas again. Rats harbouring adequate numbers of mites were kept for ten days at least from the date of capture. The engorged larvæ were placed in separate tubes.

At the end of ten days the rats were cardipunctured and mice inoculated with washed cells from about 1 c.c. of blood.

Mites, from rats proved positive, were separated from the others and the larvæ from both lots of mites were fed on laboratory-infected mice to observe the progress of infection.

When an infected batch of mites is developed it is hoped to compare the larval, nymphal and adult forms with the batch which so far has proved refractory to infection with *R. orientalis*.

The singular patchy distribution of endemic areas of Scrub typhus may be due in part to the presence of physiologically different races or variants of *T. deliensis*.

5. Hæmatological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

SYNOPSIS.

Nutritional macrocytic anæmia.—Dietary inadequacy, and hypersplenism singly or in combination appear to be contributory factors for the precipitation of relapse. Preliminary studies indicate that normal human serum exerts a 'ripening' effect on NMA megaloblasts in bone-marrow culture. In general, initial response to citrovorum factor was favourable but the improvement was not sustained in most of the cases. With oral terramycin therapy partial clinical and hæmatological improvement was noticed in two patients with megaloblastic bone-marrow. Fæcal excretion of vitamin B₁₂, as assayed by microbiological method, dropped to zero level in both the cases during terramycin therapy.

Dimorphic anæmia.—Pathogenesis and hæmatopoiesis have been studied in detail. Greater incidence of dimorphic anæmia has been recognized. 'Hypochromic megaloblast' has been described as an indicator of dimorphic erythropoiesis.

Iron metabolism.—Serum-iron level and iron-binding capacity of serum are being estimated in different types of anæmia. These studies have proved useful in differentiating iron deficiency hypochromic anæmia from familial erythroblastic anæmia. In iron-deficiency anæmia serum iron is low, iron-binding capacity high and iron-absorption rate greatly increased. Whereas in the familial erythroblastic anæmia, serum iron is normal or high, iron-binding capacity is very low and iron-absorption rate very greatly decreased.

I. INVESTIGATION INTO CAUSES OF RELAPSE IN NUTRITIONAL MACROCYTIC ANÆMIA.

Eleven cases of nutritional macrocytic anæmia showing relapse were investigated. The criteria for relapse were progressive clinical deterioration and characteristic hæmatological changes viz. peripheral macrocytosis with megaloblastosis, and/or giant granulocytic cells and hyper-segmented inactive megakaryocytes in the bone-marrow. Details of the cases are shown in Table I ;—

TABLE I.
Details of 11 cases of *NMA*.

Serial number	Name	Age	Sex	Occupation	Econo- mic status	Average daily diet (approx.)		Gastric analysis	Infections	Enlarge ment of spleen below costal arch	Plasma bilirubin in mg. per 100 c.c.	Relapse	
						Protein intake g.	Total calories					Number	Interval
1	A.M.	28	M	Railway watch man	Poor	40	1,600	Hypochlor- hydria	...	5 cms.	2.0	1	8 months
2	Z.B.	46	F	Housewife	Poor	46	1,750	Normal	...	9 cms.	0.8	1	5 years
3	R.C.	48	M	Cultivator	Poor	45	1,712	Hypochlor- hydria	<i>P. falciparum</i> } Initially & Hookworm ova } during relapse	13 cms.	1.5	1	7 years
4	B.C.	18	M	Student	Lower middle class	48	1,538	Hypochlor- hydria	Hookworm ova } Initially	12 cms.	1.2 to 3.0	1	7 months
5	J.S.	36	M	Store keeper	Lower middle class	50	2,100	Hypochlor- hydria	1,100 Giardia cyst	...	0.2	1	6 years
6	K.G.	32	F	Refugee	Poor	40	1,800	Normal	0.2	1	2 years
7	S.C.	32	F	Housewife	Lower middle class	37.6	1,477	Normal	<i>Ascaris</i> Initially	...	0.2	2	(i) 1 yr. 3m. (ii) 3 yr. 5m.
8	K.C.P.	59	M	Clerk	Lower middle class	41.0	1,900	Normal	Hookworm ova 200-300-Initially & during two relapses	...	0.2	2	(i) 7 months (ii) 6 years
9	U.S.	28	M	Teacher	Poor	40	1,850	Normal	Hookworm ova-Initially & during 1st relapse. <i>P. vivax</i> during 2nd relapse	...	0.2	2	(i) 1 yr. 8m. (ii) 1 yr. 6m.
10	D.N.	20	M	Salesman	Lower middle class	50	1,908	Achlor- hydria } Hypochlor- hydria }	<i>Giardia</i> (cyst)-Initially and during 1st relapse. <i>Microfilaria</i> during 1st & 2nd relapses	10 cms.	1.0 to 2.0	2	(i) 1 year (ii) 10 months
11	S	23	M	Railway watch man	Poor	44	1,708	Achlor- hydria } Hypochlor- hydria }	<i>P. vivax</i> } Initially <i>microfilaria</i> } <i>Ascaris</i> ova 700-2,200 during 2nd & 3rd relapses	...	0.6	3	(i) 4 years (ii) 1 year (iii) 7 months

Of the eleven patients, one patient relapsed thrice ; four patients relapsed twice and the remaining six relapsed once. Minimum interval between two attacks was seven months and the maximum interval was seven years.

Age.—This varied from 18 to 59 years. The distribution in different age groups was as follows :—

<i>Year</i>		<i>Cases</i>	
18-29	5
30-40	3
41-59	3

Sex.—Eight males and three females.

Occupation and environmental factors.—Nothing incriminating was found in any case. The male patients were comprised of cultivators, labourers, clerk, school teacher and student. The female patients were housewives.

Economic status.—Five patients belonged to lower-middle class and the remaining six were poor.

Diet.—Four were vegetarians and seven used to a mixed diet. Diet was inadequate in protein and fat in all the cases. Approximate protein content of daily diet varied from 37·6 g. to 50·0 g. In spite of pointed instructions for supplementing the diet the patients failed to improve their diet due to poor economic condition.

Gastric hydrochloric acid.—Normal acid curve was obtained in five patients. Four patients showed varying degrees of hypochlorhydria. Two patients showed absolute achlorhydria at the height of anæmia ; in both of them the achlorhydria was reversible, acid returning along with clinical and hæmatological improvement.

Infection.—Seven patients showed evidences of parasitic infections, viz. malaria, hookworm, ascaris, giardia and filaria. Hookworm ova (varying from 200 to 1,100 per gramme of stool) were found in four patients ; in three cases hookworm infestation was associated with other infections, viz. malaria in two and giardia in one. During anti-anæmic therapy malarial parasites were detected in three patients (*P. vivax* in two and *P. falciparum* in one) ; one patient gave past history of malaria. Cystic forms of giardia were found in two patients. Ova of *Ascaris lumbricoides* were found in two patients.

Presence of a hæmolytic component and the rôle of hypersplenism.—Five patients had greatly enlarged spleens (8 cm. to 12 cm. below costal margin) with varying degrees of hyperbilirubinæmia (0·8 mg. to 3·0 mg. per 100 c.c.). Basic reticulocyte count, however, was not high in all the cases. One of these patients gave history of malarial infection and one patient had an attack of malignant tertian malaria.

Two patients in this group had a basic anomaly of erythropoiesis analogous to, if not identical with, hereditary leprocytosis. Basic dystrophy of red cells was initially masked by AMF deficiency. With the correction of AMF deficiency, characteristic hypochromic blood picture with increase in number of target cells and normoblasts in the peripheral blood pointed to the basic erythropoietic anomaly. Characteristic features in these two cases were (1) tremendous erythropoietic overactivity, much more than what is usually seen in an uncomplicated case of NMA ; (2) sub-optimal response to

AMF therapy. AMF therapy corrected the megaloblastosis and macrocytosis but did not normalize the blood picture which at the conclusion of AMF therapy showed features of hereditary leptocytosis. One of these cases, after a second relapse, was splenectomized in 1949. The result of splenectomy was reduction of erythropoietic over-activity of bone-marrow and stabilization of the blood at a subnormal level. This patient has not shown any further relapse of NMA. Basic erythrocytic dystrophy was, however, unaltered and since splenectomy degree of hypochromia, leptocytosis and normoblastosis had increased. The other patient who had a second relapse after ten months (first relapse occurring after the lapse of one year) is at present under investigation.

It is likely that an enlarged spleen through hypersplenic mechanism (either by bone-marrow inhibition or by accelerated erythrophagocytosis or erythrolysis) may interfere with utilization of AMF, and this process may not only precipitate a relapse but may also limit the expected response to AMF therapy.

Rôle of steatorrhœa.—Fat-balance studies were done according to the method of van de Kamer *et al.* (*Jour. Biol. Chem.*, **177**, p. 347, 1949). The total daily fat intake during a four-day period varied from 40 g. to 80 g. In the patient who showed three relapses the average daily fœcal output was 24 per cent (normal patients under this régime excrete six to ten per cent). With improvement of blood picture the output was reduced to 6.06 per cent. The other two patients showed daily fœcal outputs of 20 per cent and 21 per cent which after the improvement of anæmia fell to ten per cent and 12 per cent, respectively.

Two patients showed little or no absorption of ingested glucose as revealed by standard glucose-tolerance test; one patient showed decreased absorption. Absorption patterns improved along with clinical and hæmatological improvement.

During this period three other patients showing relapse represented cases of Addisonian pernicious anæmia. One was an elderly Anglo-Indian aged 79 years, one Jewess aged 65, and the third an elderly Hindu widow aged 59 years. The last case was reported in 1951 along with two other patients (pernicious anæmia in Asiatic Indians—Das Gupta, C. R., and Chatterjea, J. B., *Blood*, **6**, p. 631, 1951). All these patients had constant achlorhydria and presented evidences of sub-acute combined degeneration of spinal cord.

II. ANTIBIOTICS IN NMA WITH REFERENCE TO EXCRETION OF VITAMIN B₁₂, AS ESTIMATED BY MICROBIOLOGICAL ASSAY.

(i) *Effect of terramycin on hæmatological and clinical picture.*—Terramycin in oral dosage of 1 g. to 1.5 g. daily for ten days was given to two patients. One patient received a total dosage of 15 g. and the other received 12.5 g. According to the formula of Della Vida and Dyke the improvement index in both cases, calculated over a two-week period, was 44 per cent of the expected rate of improvement. In one case (J.S.) the improvement continued for a further period of three weeks. In the other case (K.B.D) improvement was not sustained; this patient later improved with folic acid, vitamin B₁₂ and liver extract. The maximum improvement of blood picture in both the cases after terramycin therapy is shown below in a tabular form. The first patient was a vegetarian male and the other one was a female used to a mixed diet:—

III. LEUCOVORIN IN NUTRITIONAL MACROCYTIC ANÆMIA.

Leucovorin is a synthetic product related chemically to folic acid. Its structural formula is 5-formyl 5, 6, 7, 8, tetrahydropteroyl glutamic acid. It is identical with citrovorum factor and folinic acid. A small supply of the product was obtained through the courtesy of Messrs. Lederle Laboratories, Ltd. Leucovorin was administered intramuscularly in 3 mg. daily dose for six days to six cases of nutritional macrocytic anæmia with megaloblastic bone-marrow. The initial response calculated according to the formula of Della Vida and Dyke was optimum in four cases ; 61 per cent of the expected response was seen in the fifth case ; no response was seen in the sixth case, a female patient aged 30 years with an obvious hæmolytic component manifested by massive splenomegaly, reticulocytosis and hyperbilirubinæmia. The sixth case later on showed much better response with folic acid. The improvement was not sustained in three of the cases, showing optimum initial response.

IV. *IN VITRO* CULTURE OF BONE-MARROW IN NUTRITIONAL MACROCYTIC ANÆMIA.

The method employed was that of Osgood (Osgood, E. E., and Brownlee, I. E., *Jour. Amer. Med. Assoc.*, **108**, p. 1793) as modified by Lajtha (*Jour. Clin. Path.*, **5**, p. 67, 1952).

(i) 2 c.c. of marrow collected with sterile technique was mixed with 18 c.c. to 20 c.c. of Gey solution (Gey, G. O. and Gey, N. K., *Amer. Jour. Cancer*, **27**, p. 45, 1936) in a sterile McCartney bottle kept at 30° C. to 36° C. Within one hour the bottle was centrifuged for ten minutes at 1,500 r.p.m.

(ii) The supernatant fluid was pipetted off under sterile precautions. 5 c.c. to 10 c.c. of Gey's solution was added to the cell suspension and mixed thoroughly.

(iii) A total nucleated cell count of the mixture was done and necessary adjustments were made to obtain a final count between 15,000 to 50,000 per c.mm.

(iv) The final culture medium was prepared by mixing proper proportions of Gey's solution and compatible normal human serum in such a way that the total nucleated cell count of the mixture was between 2,000 to 4,000 per c.mm. Three c.c. samples of the final mixture were injected into each of a number of McCartney bottles. Basic total and differential counts were made on this sample before incubation. These represented the '0 hour' count. Culture bottles were then kept in an incubator at 37° C., and at desired intervals a bottle was taken out and total and differential counts made.

The behaviour of megaloblasts in NMA was studied *in vitro*. Preliminary results show that normal human serum of compatible blood group has a 'ripening' effect on megaloblasts and a maturing effect on normoblasts. Results of a typical experiment where megaloblastic bone-marrow was cultured in a medium containing equal parts of normal serum and Gey's solution are shown below in Table III :—

TABLE III.

Data on bone-marrow culture from a case of NMA.

Hours	Total nucleated-cell count per c.mm.	PERCENTAGE PER 100 NUCLEATED RED CELLS							Transi- tional
		Pro-ery thro- blast	Megaloblast			Normoblast			
			A	B	C	A	B	C	
0	3,900	1.0	3.0	6.0	3.0	12.0	12.0	61.0	2.0
22	3,800	0.0	0.0	0.8	3.0	0.0	7.0	87.2	2.0
27	3,200	0.0	0.0	0.0	3.0	0.0	6.0	90.0	1.0
48	3,750	0.0	0.0	0.0	0.0	0.0	1.0	99.0	0.0

V. DIMORPHIC ANÆMIA.

Clinical and hæmatological investigations were carried out in 103 unselected cases of deficiency anæmias with a view to find out the incidence of dimorphic anæmia (DA). A detailed account of the findings has already been published (*Ind. Med. Gaz.*, **88**, p. 126, 1953), the salient findings are only reported here :—

(1) *Incidence* of DA in the present series was 51.4 per cent. Deficiency of both antimegaloblastic factor (AMF) and iron was of considerable degree in 51 per cent ; deficiency of AMF was predominant in 38 per cent and deficiency of iron was predominant in 11 per cent of cases.

(2) DA has no characteristic clinical features by which it can be differentiated from NMA and iron, deficiency anæmia.

(3) In the present series, 83 per cent presented a *macrocytic hypochromic* blood picture : 7.6 per cent had macrocytic orthochromic, 5.6 per cent normocytic hypochromic and 3.8 per cent normocytic orthochromic blood picture.

(4) *Diagnostic features :*

(i) *In peripheral blood smear.*—

- Association of obviously macrocytic cells with grossly hypochromic red cells.
- Presence of macropolycytes and hypochromic red cells in the same smear.

(ii) *In bone-marrow.*—

- Hypochromic megaloblasts.
- Association of hypochromic normoblasts with giant metamyelocytes, giant stabs and macropolycytes.

(5) Important factors in the pathogenesis of DA were :—

- Dietary inadequacy of substances, such as meat, liver, egg which are rich sources of both AMF and iron.
- Dietary inadequacy associated with hookworm infestation or other causes of hæmorrhage.
- Gastro-intestinal disorders, such as achlorhydria, diarrhœa, dysentery and steatorrhœa.

Present investigations indicate that dimorphic anæmia is a distinct hæmatological entity.

VI. STUDIES ON IRON METABOLISM.

Serum iron was determined according to the Powell's modification of Heilmeyer's method and iron-binding capacity according to the method of Shade and Caroline (1946) as modified by Cartwright and Wintrobe (*Jour. Clin. Invest.*, **28**, p. 86, 1949). Rate of iron absorption was studied by giving a test dose of 12 grains of ferrous sulphate by mouth and noting the rise in serum-iron level two and six hours after the test dose. The patterns of serum-iron level, total iron-binding capacity of serum, per cent saturation and intestinal absorption rates of iron in different types of anæmias were as follows. All these tests were repeated after the specific therapy had improved the blood picture.:—

(i) *Hypochromic anæmia (three cases)*.—The mean serum iron (S.I.) in this group was 20.6 μ g. with a range varying from 17 to 38 micrograms. Unsaturated iron-binding capacity of serum (U.I.B.C.) ranged from 325 μ g. to 500 μ g. with a mean of 400 μ g. Iron-saturation values ranged from 3.4 to 9.2 per cent with a mean of 6.5 per cent. Following the test dose of iron the S.I. showed a mean maximum increase of 143.5 μ g. over the basic level. Thus, in this group the S.I. and iron saturation were low, U.I.B.C. increased and absorption rate exaggerated. After a course of iron therapy for one month when the blood picture had nearly reached normal levels, the tests were repeated. Repeat tests showed rise in S.I., rise of iron saturation and reduction of U.I.B.C. The mean values for S.I., U.I.B.C. and per cent saturation at the end of therapy was 86 μ g., 365 μ g. and 19.2 per cent, respectively. Rate of iron absorption was, however, still greatly increased, the mean maximum increase of S.I. following the test dose being 251.5 μ g., over the basic level.

(ii) *Dimorphic anæmia (eight cases)*.—Before treatment the mean S.I. was 20.0 μ g. (range 5 to 29), mean U.I.B.C. was 379 μ g. (range 325 to 500) and mean iron saturation was 4.6 per cent (range 1.5 to 7.1 per cent). Following the test dose the mean maximum rise in S.I. level was 273 μ g. over the basic level. The pattern in this group thus closely resembled that of hypochromic group.

After improvement of blood picture with iron and liver extract both the S.I. and iron saturation increased and the U.I.B.C. showed proportionate decrease. Mean values of S.I., U.I.B.C., and iron saturation at this stage was 62.6 μ g., 338.0 μ g. and 15.5 per cent, respectively. Rate of iron absorption was, however, still increased, the mean maximum increase of S.I. following the test dose being 146.0 μ g. over the basic level.

(iii) *Familial erythroblastic anæmia (five cases)*.—Mean S.I. level in this group was 162.60 μ g. (range 100 to 263). U.I.B.C. ranged from 0 to 166 with a mean of 63.3 μ g. Iron saturation ranged from 42.9 per cent to 100 per cent with a mean of 72.3 per cent. Absorption rate following a test dose of iron showed a mean increase of only 37 μ g. over the basic level. Thus, in this group S.I. was high (or normal), iron saturation greatly inflated, U.I.B.C. greatly reduced and absorption rate considerably decreased. The different values were unaffected by any therapy.

(iv) *Aplastic anæmia (four cases)*.—Mean S.I. was 247.0 μ g. (range 100 to 400). Mean U.I.B.C. was 129.0 μ g. (range 0 to 238) and mean iron saturation was 69.5 (range 41.9 to 100) per cent. In this group S.I. was high, U.I.B.C. low and iron saturation greatly increased.

(v) *Nutritional macrocytic anæmia (nine cases).*—Mean value for S.I. was 137.1 (range 80-225). Mean U.I.B.C. was 168.8 (range 50-300) and the mean iron saturation was 46.8 (range 31.3 to 81.8) per cent. Following the test dose, the mean maximum increase of serum iron was 62.5 μ g. over the basic level. Thus, in NMA serum iron was normal (or high), iron-binding capacity low, iron saturation increased and absorption rate decreased.

After improvement of blood picture mean S.I. level was 72.5 μ g., mean U.I.B.C. was 347.6 μ g. and mean iron saturation was 17.2 per cent.

(vi) In anæmia due to infection, serum iron was low and iron-binding capacity depressed.

The findings in different groups are shown in Table IV. These studies have proved useful in differentiating hypochromic anæmia from anæmia of infection and familial erythroblastic anæmia.

TABLE IV.

Serum iron, iron-binding capacity, iron-saturation and iron-absorption patterns in different types of anemia.

Type of anemia	SERUM IRON (S.I.) IN μ g./100 C.C.				UNSATURATED IRON-BINDING CAPACITY OF SERUM (U.I.B.C.) IN MICROGRAM/100 C.C.				IRON SATURATION IN PER CENT				MAXIMUM RISE OF SERUM IRON OVER BASIC LEVEL FOLLOWING A TEST DOSE OF GR. 12 OF FERROUS SULPHATE			
	Before treatment		After treatment		Before treatment		After treatment		Before treatment		After treatment		Before treatment		After treatment	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Hypochromic anemia	20.6	17.0 to 38.0	86.0	63.0 to 109.0	400.0	325.0 to 500.0	365.0	330.0 to 400.0	6.5	3.4 to 9.2	19.2	13.6 to 24.8	143.5	87.0 to 200.0	251.5	162.0 to 341.0
Dimorphic anemia	20.0	5.0 to 29.0	62.6	48.0 to 86.0	379.0	325.0 to 500.0	338.0	300.0 to 400.0	4.6	1.5 to 7.1	15.5	11.7 to 17.7	273.0	90.0 to 422.0	146.0	74.0 to 325.0
Familial erythroblastic anemia	162.6	100.0 to 263.0	63.3	0 to 166.0	72.3	42.9 to 100.0	37.0	12.0 to 50.0
Aplastic anemia	247.0	100.0 to 400.0	129.0	0 to 238.0	69.5	41.9 to 100.0
Nutritional macrocytic anemia	137.1	80.0 to 225.0	72.5	50.0 to 100.0	168.8	50.0 to 300.0	347.6	300.0 to 400.0	46.8	31.3 to 81.8	17.2	12.5 to 21.7	62.5	50.0 to 75.0

VII. CLINICAL TRIAL OF LIVER EXTRACTS RECEIVED THROUGH I.C.M.R.

An oral preparation containing extracts of both liver and stomach was assayed by clinical trial in suitable cases of NMA. In the dosage advocated by the manufacturer the preparation was effective but did not bring the blood picture to normal level. The detailed report was submitted to the Secretary, I.C.M.R.

VIII. STANDARDIZATION OF HÆMATOLOGICAL INSTRUMENTS MANUFACTURED IN INDIA.

During the period under review no samples were received for standardization.

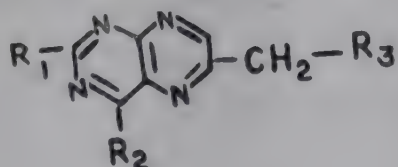
6. Inquiry on the synthesis of anti-folic acid compounds and studies on their chemotherapeutic activities under Dr. S. C. Roy at the University College of Science & Technology, Calcutta.

SYNOPSIS.

Nine anti-folic acid compounds have been synthesized so far and their characteristics determined by the measurement of the ultra-violet absorption spectra. The anti-folic acid activity of some of the synthesized compounds has been determined by the microbiological assay method with *S. faecalis* R as the test organism. The testing of one of the potential compounds against leukemic strain of mice is under way.

In addition to the synthesis of seven anti-folic acid compounds as reported before, another two new analogues have been synthesized during the period under review, over and above the determination of the antagonistic activity of some of the synthesized compounds by the microbiological assay method, using *S. faecalis* R as the test organism and establishing the characteristics of those compounds through the measurement of their absorption spectra with the Beckman spectro-photometer, model 'DU'.

The following two additional compounds have been synthesized.



Compound I— $R_1 = \text{NH}_2$, $R_2 = \text{OH}$, $R_3 = p\text{-arsanilic acid}$

Compound II— $R_1 = \text{NH}_2$, $R_2 = \text{NH}_2$, $R_3 = p\text{-arsanilic acid}$

The method of preparation was very similar to that of Waller *et al.*, (*Jour. Amer. Chem. Soc.*, 1948, **70**, p. 21).

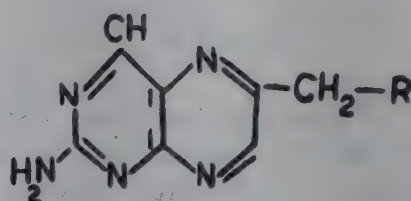
Equimolecular quantities of *p*-arsanilic acid and the pyrimidine [2, 4, 5-triamino 6-hydroxypyrimidine in case of (I) and 2, 4, 5, 6-tetraaminopyrimidine in case of (II)] were dissolved in water and to it dibromopropionaldehyde dissolved in ethanol and sodium di-chromate solution (both in the same mol. proportion) added drop by drop with thorough

stirring of the mixture. The pH and the temperature of the solution were maintained, at 4 and 45° respectively. When the reaction was complete, the precipitate was cooled and filtered.

The crude material obtained in the above manner was dissolved in NaOH solution, clarified with charcoal at pH 8 and precipitated by diluted HCl at pH 4. By repeating the above procedure three times, pure crystalline analogues were obtained.

Ultra-violet absorption spectra of the synthetic analogues.—The ultra-violet absorption spectra of 10 mg./litre solution of the synthetic analogues in 0.1 N NaOH and 0.1N HCl were determined in 1 cm. cell at 5 m μ . intervals on a Model 'DU' Beckman spectro-photometer using a solvent filled cell in the reference position. The per cent transmittance values were measured at each wave length. Additional data were obtained at 1 m μ . intervals at the points of inflections. The ultra-violet absorption maxima and minima in m μ . of the substances in 0.1N NaOH and 0.1N HCl are given below :—

Ultra-violet absorption of the analogues in m μ .



R =	Ultra-violet absorption in m μ .			
	0.1N NaOH		0.1N HCl	
	Maximum	Minimum	Maximum	Minimum
<i>m</i> -toluidine ...	252,360	230,305	319	275
<i>p</i> -amino-methyl-benzene sulphonamide ...	252,361	230,302	318	274
<i>p</i> -amino-phenyl acetic acid ...	252,360	230,299	315	270
<i>p</i> -amino-phenyl acetyl glutamic acid ...	255,364	226,312	317	275
<i>p</i> -amino-salicylyl-glutamic acid	232,325	310

The above compounds were oxidized with alkaline permanganate to 2-amino-4-hydroxypteridine-6-carboxylic acid and the identity was proved by comparing its ultra-violet spectrum with that of an authentic sample. This reaction proved that the connection of the side chains to the pteridine ring were in the 6-position. The analogues of folic acid which have been synthesized were tested microbiologically for their antagonistic activity to folic acid against *S. faecalis* R. The inhibition index which is defined as the ratio by weight of the inhibitor to that of pteroyl-glutamic acid at which half inhibition of growth of the organism occurs was determined for different analogues.

The micro-organism used in the experiment was *S. faecalis* R. (ATCC 8043). The medium used for the growth was adapted from that given in the 'Methods of Analysis' (Association of Official Agricultural Chemists, 7th ed., p. 734, 1950) and described in details in the 'Methods of Vitamin Assay'.

The general procedure was to set up a series of tubes with a constant amount of pteroyl-glutamic acid (5 m μ . per tube for *S. faecalis* R.) and varying amounts of inhibitor. After a definite period of incubation the growth was measured turbidimetrically in the 'Klett-Summerson' photo-electric colorimeter using the 650-m μ . filter, and the point at which half inhibition of the growth occurred was obtained. The results are given in the Table :—

TABLE.

Inhibition indices of the synthetic analogues of pteroyl-glutamic acid.

R =	Inhibition index
1. <i>m</i> -toluidine	7,000
2. <i>p</i> -amino-phenyl acetic acid	26,000
3. <i>p</i> -amino-phenyl acetyl glutamic acid	1,200
4. <i>p</i> -amino-salicylyl glutamic acid	280
5. <i>p</i> -amino-phenyl arsinic acid	10,000

While 4-aminopterin which is a highly antagonistic compound and being therapeutically used against leukemia has got an inhibition index of 2.66 (Smith *et al.*, *Trans. N.Y. Acad. Sci.*, II, 1948, **10**, p. 82).

**7. Inter-laboratory evaluation of procedures for serological tests for syphilis followed in various laboratories in India under
Dr. R. V. Rajam, Director, Upgraded V.D. Department,
Government General Hospital, Madras.**

An essential feature of this programme is that all participating laboratories will employ the same testing procedures and use the same lots of antigens in each of the three tests. Uniformity in the method of recording of reading in the reaction tubes and in the reporting of results will facilitate compilation of the data and comparative evaluation of results. To this end, notes on technical details of the three tests procedures—qualitative, quantitative and antigens from the same batch numbers were sent to all the laboratories in advance by Dr. K. V. Venkatraman, Calcutta. Further, four blank forms for recording detailed informations on (1) donors, (2) serum specimens, (3) reagents, and (4) for reporting of results were printed in adequate numbers and forms III and IV distributed in advance to all the laboratories, while forms I and II were retained at Madras. The reagents other than antigens could not be issued from one source and such individual laboratories provided them from their own resources, however, giving complete details regarding them.

Donors for serum samples were selected with a view to obtain, as far as possible, a definite percentage of several categories of sera, including those from syphilitics in various clinical stages treated and untreated, normal healthy persons and from diseases other than syphilis. The V.D. clinic and other units of the General Hospital, Madras, formed the sources

of the donors, paid and unpaid for this inquiry. Adequate informations on donors and serum specimens were entered on forms I and II, respectively, and kept at Madras with Dr. R. V. Rajam. Twelve donors were bled on Fridays of a week, as far as possible ; 50 c.c. to 60 c.c. of blood were taken from the vein before a meal from each donor into 200-c.c. test-tubes and allowed to clot. About 20 c.c. to 25 cc. of clear serum was obtained by centrifugation of the serum separated from the clot. Sterile precautions were observed throughout ; however, merthiolate was added to each sera in 1 in 10,000 strength and preserved in the refrigerator with appropriate code numbers until ready for despatch. The strength of the merthiolate was later increased to 1 in 1,000 as some laboratories reported ' contamination ' on reaching them. On Tuesday of a week the sera were distributed in 2-c.c. to 3-c.c. quantities in 5-c.c. vials stoppered securely with corks. One vial of each serum was then sent as ' unknown ' to each participating laboratory outside Madras by air parcel in empty tin-containers safely packed. The remaining vials from each specimen of sera were stored in the freezing compartment of the refrigerator and despatched with code numbers, one a time to a designated laboratory at intervals of seven days along with regular specimens on Tuesdays. The object was to get each test repeated at least three times by the participating laboratories on a proportion of serum samples for the reproducibility of test results at intervals of seven days. All laboratories had been instructed to perform their tests on the same day, namely Fridays of a week, and the unknowns were put in the air mail on Tuesday to reach their destination at least by Thursdays of the week. The two Madras laboratories kept their quota of samples at room temperature until Friday so as to simulate the condition under which the sera were in transit to outside laboratories.

The results of the tests obtained from each test and the information on reagents used by the participating laboratories were entered in triplicate in the printed forms III and IV and a copy of each was sent to Dr. R. V. Rajam at Madras and Dr. K. V. Venkatraman at Calcutta, and the third copy retained by the Laboratory for its own record.

By the 30th of September report on 170 sera was received from all the laboratories except Agra which joined the inquiry on 20th August, 1953, and hence had reported only on 59 sera. The donors included the following categories :—

1.	Clinical syphilis	78	46·43 per cent.
	Primary syphilis	24	
	Secondary syphilis	27	
	Latent	10	
	Late benign	6	
	Cardiovascular syphilis	3	
	Neurosyphilis	5	
	Congenital syphilis	3	
2.	Normal	30	17·26 per cent (1 rejected)
3.	Venereal but non-syphilitic	15	9 per cent
4.	Diseases other than of venereal origin	47	27·31 per cent (1 rejected)
Total				170	(2 rejected)

This included the following :—

Tropical eosinophilia	5
Leprosy	7
Malaria	2
Visceral leishmaniasis	3
Upper respiratory	5
Upper respiratory infections	5
Herpes pro-genitalis	3
Pregnancy	6
Vitiligo	1
Granuloma annulare	1
Filariasis	2
Cancer	2
Rheumatoid arthritis	1
Impetigo	1
Warts penis	3
Psoriasis	1
No appreciable disease	3

There has been some difficulty in obtaining enough donors in unequivocal diagnostic categories indicated in this inquiry. A good beginning has, however, been achieved.

Qualitative and quantitative tests were performed on all specimens using uniform procedure by all the three tests from all the laboratories. V. D. Laboratory, Madras, participated in the inquiry unofficially since only Kahn and V.D.R.L. tests were entered for want of facilities for complement-fixation test here. There were a few instances of breakages and of quantity not sufficient for quantitation. There was some initial confusion and a few irregularities in the performance of the tests and reporting of tests but later on there was improvement in carrying out the instructions by all the laboratories. Twenty-one sera were sent to each laboratory for repeat tests, most of them three times. The intervals between each repeat test could not be kept seven days to begin with. It varied from one day to six weeks during which time the specimens were, however, kept frozen solid. A definite proportion of reactive and non-reactive specimens sent for repeat test to each laboratory could not also be made. Arrangements are, however, in hand at present to rectify these drawbacks. The inquiry could not be started earlier than 1st June, 1953, owing to unavoidable delays in the procuring of equipment, laboratory facilities and personnel.

A standard serological test for syphilis to be considered as having satisfactory test performance should embody all the following three basic requirements at the same time : (1) Sensitivity, (2) specificity and (3) reproducibility of test results.

Keeping these in view, a preliminary analysis of results received on 170 sera and repeat tests on 21 specimens out of them has been attempted. No definite conclusions are drawn at this stage from the analysis as the number of sera examined under the various diagnostic categories is too small to be significant. It must, however, be said there is scope for improvement in the technical performance of all the three tests by the laboratories taking part in the inquiry.

8. Clinical research unit under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

SYNOPSIS.

Chemotherapy of malaria.—Further clinical trials with paludrine showed increased number of refractory cases including 1 *vivax* infection. Mosquitoes fed on two patients during paludrine treatment were found to develop heavy sporozoite infection of salivary glands and to transmit the disease, thus disproving the claim that the gametocytes during paludrine treatment are non-infectious mosquito. Daraprim often failed to cure *falciparum* cases. Both paludrine and daraprim are, therefore, not considered very suitable for the treatment of acute *falciparum* malaria. Primaquine with schizonticidal drugs tried in a larger number of cases was found to reduce relapses in *vivax* malaria to about ten per cent, the lower toxicity being an advantage over pamaquine. Daraprim in weekly doses of 25 mg. and camoquin in fortnightly doses of 0.4 g. were found to be effective suppressives. Mepacrine in a single dose of 0.6 g. gave encouraging results, but early relapse might occur. Compound C had no advantage over camoquin. Nivaquine by intramuscular injection was found to be effective and less painful than quinine.

Tropical splenomegaly.—Investigations were carried out into the pathogenesis of the condition. Forty cases were admitted to the hospital for the purpose. The history indicated a *relationship with malaria* and parasites were found in three cases—two after splenectomy. No Rh incompatibility was found. Spleno-portal venography showed dilatation and tortuosity of splenic and portal veins, visualization of finer tributaries in a few cases—the dye having entered against the usual direction of blood flow and rarely thrombosis of splenic vein. The adrenaline test gave evidence of marked hypersplenism but in case of huge splenomegaly the contraction was poor due to the extensive fibrosis. This was, however, a more favourable condition for sequestration and phagocytosis of the formed elements of blood. The biopsy specimens of liver showed absence of hepatic pathology in the majority but some cases showed foci of degenerated hepatic cells, and fibrosis or increased reticulin formation was detected in a few.

White rats infected with *P. berghei* which spontaneously recovered (parasite negative) from the infection showed after an interval of two to three months a residual splenic enlargement to about double the normal size. This experimental observation has been started to see if malarial infection in rats may give rise to this type of splenomegaly.

The results so far obtained suggest that the cases of 'splenomegaly' under investigation is a sequel of repeated attacks of malaria leading to hypersplenism with consequent anæmia, leucopænia, etc., usually without any primary structural change in the liver.

Epidemic dropsy.—A simple method has been developed for the isolation of total alkaloids directly from argemone seeds. The acute and chronic toxic effects of the total alkaloids as hydrochloride have been studied in rats, mice and rabbits. The alkaloids were found to

induce increased capillary permeability using the trypan-blue technique of Menkin. The technique for the direct observation of capillaries in the rabbit's ear (modified Sandison-Clark chamber) is being developed to study the toxic effect of alkaloids on blood vessels. Some drugs, viz. phenergan, ephedrine, British anti-Lewisite, cortisone, rutin and vitamins were used to counteract the acute and chronic toxic effect of the oil and alkaloids. Only BAL could afford some protection from the lethal effect of the toxin but could not prevent the increase of capillary permeability. Cortisone enhanced the lethal effect of the toxin and abolished the protective action of BAL.

More samples of 'white oil' were found to be toxic to mice, rats and rabbits. White oil 'purified' by a chemical process did not reduce its toxic action. Cutaneous application of white oil was found to be very toxic to rats producing a lethal effect within four or five days.

Cholera.—The changes in the plasma sodium and potassium in cholera were investigated by Flame-photometer which showed high initial potassium level usually coming down to normal or sub-normal values with treatment; but in uræmic cases the potassium level was persistently high probably contributing to their fatal termination. Such abnormal level of potassium has a very important bearing on the prognosis and treatment.

Further trials with intravenous chloromycetin showed that this drug while rapidly clearing the stools of vibrios had no significant effect on the clinical course or mortality, thus confirming our preliminary observation.

Antihistaminics and vitamin C were not found to be of any value in preventing or curing uræmia in cholera, as recently claimed.

MALARIA.

A. Chemotherapy.

1. *Recent observations on treatment with paludrine.*—High hopes raised about paludrine when it was introduced have not materialized. Our extensive trials have shown that there are wide variations in its efficacy against different strains of malaria parasites, that it has a relatively slow action which makes it unsuitable for acute *falciparum* infection and that it tends to induce the emergence of drug-resistant variants of the parasite. Such strains now appear to be more common than previously. Ten malaria cases (four *vivax*, five *falciparum*, one mixed *falciparum* and *vivax*) admitted to the hospital were recently treated with paludrine 0.6 g. on 1st day and 0.3 g. daily for next four to five days. The response was slow and four of the six cases with *falciparum* infection had recrudescence of malaria within 12 days. Paludrine was again used in two of these. One failed to respond for four days when another anti-malarial was used and the other (mixed infection) responded very slowly (when the dose was doubled and continued for ten days) taking five days to be afebrile and nine days to be free from parasites of both the species.

It has been claimed that the gametocytes present in the patient's blood are non-infective to mosquitoes during paludrine treatment. Our

recent observations, however, clearly show the failure of paludrine to prevent development of gametocytes in the mosquitoes. Two batches of laboratory-bred *Anopheles stephensi* were fed separately on two *falciparum* cases with crescents in the peripheral blood, while the patients were on paludrine treatment. The urine of both the patients showed the presence of paludrine at the time of the mosquito feeds. Two to three weeks later some of the mosquitoes in both the batches showed heavy sporozoite infection of the salivary glands. Two mosquitoes from one batch were re-fed on a volunteer who after an incubation period of 11 days developed a clinical attack of malaria with *falciparum* parasites in blood. Thus, the presence of paludrine in blood does not prevent the full development of gametocytes in mosquitoes and the transmission of the disease.

2. *Single-dose treatment with mepacrine*.—Mepacrine is slowly excreted from the body. It was, therefore, thought likely that it might be effective in a single dose for the clinical cure of malaria in the same way as chloroquine or camoquin. The trial was carried out recently on 15 cases of malaria (14 *vivax*, one *falciparum*). The age of the patients varied from three to 43 years and 13 were males and two females. The dosage schedule according to age up to five years was as follows : 0.2 g., six to ten years ; 0.3 g., 11 to 15 years ; 0.4 g. 0.6 g. above 15 years. In all the 15 cases fever was controlled and asexual parasites disappeared from the peripheral blood in two to three days, time. In three patients where the drug was given in the afebrile period subsequent paroxysms of fever were suppressed. They could be observed for six weeks only, and of them two (*vivax* infection) had recrudescence after three weeks. If confirmed by further trials this will be a cheap and effective form of remedy for malaria in semi-immune people suitable for rural dispensary practice.

3. *Radical cure of vivax malaria : Trials with primaquine*.—Primaquine or SN13272, the latest member of the 8-aminoquinoline group of drugs was tried in 44 cases of malaria (4 *falciparum*, 36 *vivax*, 2 *malariae* and 2 mixed *falciparum* and *vivax*) and the results (41 cases) were published in the *Ind. Jour. Med. Sci.*, 7, p. 236, 1953. It was used in doses of 10 mg. to 15 mg. daily for 10 to 14 days alone (17 cases), with chloroquine (0.75 g. base on first day, 0.25 g. on second and third days—ten cases), with quinine sulphate (1 g. to 1.5 g. daily for seven to ten days—16 cases) and with paludrine (0.3 g. daily for five days—one case).

Primaquine alone could terminate an acute attack of *vivax* or *malariae* infection but a more prompt response was obtained by the concurrent use of quinine or chloroquine. The combined treatment caused disappearance of fever and asexual parasites in two days in 24 out of 26 cases and in three days in the remaining two. Its action on *falciparum* malaria was slow and uncertain. *Vivax* and *malariae* gametocytes were eliminated within five days in all the cases ; crescents disappeared within four days after clearance of asexual parasites. Twenty *vivax* and one *malariae* cases—seven treated with primaquine alone (A), six with primaquine and chloroquine (B) and eight with primaquine and quinine (C) were followed for 1½ to 16 months (one and a half to two months—eight, three to six months—four, 10 to 16 months—nine (during which two patients in the last regimen (C) relapsed (one parasitic, one clinical) ; re-infection, however, was possible. Toxic action, such as nausea, vomiting abdominal pain was observed in one case,

Other workers have reported a relapse rate of about 10 to 20 per cent in *vivax* malaria treated with 2 g. quinine and 30 g. pamaquine daily for ten days. The results with primaquine (9.5 per cent relapse), therefore, appear to be comparable to the best that can be obtained with quinine-pamaquine. The drug is also preferable for its lower toxicity and lower effective dose.

4. *Daraprim*.—The results of treatment of 62 cases were analysed and published in *Jour. Ind. Med. Assoc.*, **22**, p. 155, 1953. At first a single dose of 50 mg. was used in 19 patients but as this was found ineffective in some two such doses were given on consecutive days for 43 cases. Of the 62 patients 49 were males and 13 females, 15 being below ten years of age. Fever subsided within two days in 52.5 per cent and asexual parasites disappeared within the same period of time in 62.5 per cent. A clinical attack terminated in three days in 80 per cent of the cases. The action of the drug was, however, slower than that of chloroquine or camoquin. No toxicity was observed in the above dosage but some side reactions, such as nausea, giddiness appeared when the dosage was increased to 100 mg. daily for three days. One case of *falciparum* malaria failed to respond and nine others (seven treated with two doses of 50 mg.) had early recurrence within one to two weeks. So, altogether daraprim failed to cure ten out of 26 *falciparum* cases. One *vivax* case had recrudescence within two weeks and four others relapsed after four to twelve weeks. Though reported to be highly potent in avian and rat malaria the results in human trials have not been satisfactory in our hands.

Daraprim in paludrine refractory malaria.—Two *falciparum* cases were treated with paludrine 0.6 g. on the 1st day and 0.3 g. daily for the next five days. One of them became free from fever and asexual parasites in four days but had recrudescence eight days after treatment. The second attack failed to respond to a second course of paludrine (while urine showed presence of the drug), but daraprim in two doses of 50 mg. was effective in two days. The second case became free from asexual parasites in two days but low fever up to 99° F. to 100° F. persisted and a recurrence of paroxysm with high fever and re-appearance of asexual parasites occurred a week after treatment. The second attack was treated with two doses of 50 mg. daraprim which caused disappearance of fever in two days and asexual parasites in one day. The cases were observed for two to three weeks during which no relapse occurred.

There was apparently no cross resistance between daraprim and paludrine in these two cases. This appears to be more significant since daraprim may fail in a certain percentage of *falciparum* cases. The views about the problem of cross resistance are conflicting. Rolo (1951) observed no resistance to daraprim in strains of *P. gallinaceum* and *P. berghei* that responded little or not at all to the maximum tolerated dose of paludrine and Wilson (1952) found paludrine-resistant cases of *falciparum* malaria responsive to daraprim. On the contrary, Jaswant Singh *et al.* (1952) demonstrated high degree of cross resistance between paludrine and daraprim in similar malaria and Robertson *et al.* (1952) reported daraprim to be ineffective in paludrine-resistant *falciparum* malaria indicating the presence of cross resistance.

5. *Compound C*.—This drug was received from the Bengal Immunity Research Laboratory after a preliminary laboratory test against

P. gallinaceum, *P. relictum* and *P. berghei*. Its chemical formula is very much similar to camoquin as shown below :—

Camoquin : 4(3-diethylaminoethyl-4'-hydroxyanilino)-7-chloroquinoline. Compound C : 4 (3'-diethylaminoethyl-4 hydroxyanilino)-7-chloro-2-methyl-quinoline. The drug was tried in six cases (three *vivax*, two *falciparum*, one *malariae*) usually in doses of 0.2 g. thrice daily for three days. It was found to be effective in two to three days time in the *vivax* and *falciparum* cases, judged by its immediate effect on fever and asexual parasites. In the *malariae* case, however, a second paroxysm occurred on the third day of treatment (which was continued for six days) and the asexual parasites persisted for six days. No untoward effects were observed. The drug has no advantage over camoquin.

6. *Nivaquine*.—Nivaquine or chloroquine sulphate was used in a single dose of 0.6 g. base or four tablets in 20 cases of malaria (18 *falciparum*, one *vivax* and one mixed *falciparum*+*vivax*). Five children in the series received two or three tablets (0.3 g. or 0.45 g.). Fever was controlled and asexual parasites disappeared in 17 (85 per cent) in two days time and a clinical attack was controlled with disappearance asexual parasites in three days in 19 (95 per cent) and in four days in all. There was no toxic reactions. It will be seen that these results are not significantly different from that of chloroquine diphosphate (or Resorchin). Nivaquine-sulphate solution available in 5-c.c. ampoules containing 40 mg. per c.c. was used intramuscularly in four cases of *falciparum* and one of *vivax* malaria. One *falciparum* case with cerebral symptoms also had quinine. Three injections of 0.2 g. were given usually at 8 to 12 hour intervals. The therapeutic response was prompt in all except in the case of cerebral malaria. There was little pain (less than with quinine) and no untoward reaction.

B. Chemoprophylaxis.

Trials with daraprim weekly and camoquin fortnightly which were started last year on 600 villagers including school children has been completed. After a preliminary survey the drugs were administered from July to October or November, i.e. almost throughout the transmission season. They were given personally by one member of the Clinical Research Unit. No antimosquito measures were taken.

Daraprim.—A school was selected for the daraprim group. Alternate classes comprising 172 boys had the drug in weekly doses of 25 mg. (12.5 mg. for children below ten years) upto the end of November, 1952. The remaining boys of the school (139 in number) were given dummy tablets. The results are shown in Table I :—

TABLE I.

		Parasite and spleen rate					
		July	Aug.	Sept.	Oct.	Nov.	Dec.
Daraprim group	Parasite rate per 1,000	5.7	0	1.2	...	0.7	2.3
	Spleen rate per 100	16.1	11.6
Control group	Parasite rate per 100	3.6	3.7	8.6	...	6.6	9.2
	Spleen rate per 100	16.2	23.9

It will be seen that while the parasite rate in the control group rose from 3·6 per cent in the month of July to 8·6 per cent and 6·6 per cent in September and November the corresponding rates in the treated group were 5·7, 1·2 and 0·7 per cent, respectively. Daraprim in a dose of 25 mg. weekly, therefore, appears to be an effective suppressive drug in semi-immune people. All the cases who showed parasites during treatment were, however, infected with *P. falciparum* apparently due to a relatively poor effect on this infection.

Camoquin.—Another village including two schools was selected for the purpose. There were 260 people in the treated group and 69 in the control group. The initial spleen rate in the two groups was 58 per cent and 54 per cent, respectively. The drug was administered in dose of 0·4 g. base (0·2 g. for children under ten years) fortnightly up to October 1952. The parasite rate is shown in Table II :—

TABLE II.

	Parasite rate				
	July	August	September	October	November
Camoquin group	12·5	0	0	...	4·6
Control group	11·6	10·6	23·6	...	15·1

These results confirm our preliminary observations that fortnightly doses of camaquin afford considerable protection against malaria.

TROPICAL SPLENOMEGALY.

The object of the work was to investigate into the ætiology and pathogenesis of the diseases.

Forty cases of 'tropical splenomegaly' have so far been studied as indoor patient. Apart from clinical observations, investigations were carried out into the conditions of the spleen and liver and their vascular system. For this purpose the adrenaline test, hepatic-function test, and biopsy and spleno-portal venography were performed. The structure of spleen was studied in the splenectomized cases. The hæmatological changes and any possible rôle of Rh factor were also observed.

In the group of cases studied the average splenic enlargement below the costal margin was 6·2" and the liver was enlarged up to 2½" below the costal margin in all except two cases. Ascites was present in seven cases and a history of gastro-intestinal hæmorrhage in five.

1. *Blcod picture*.—Varying degree of anæmia and leucopenia (chiefly due to neutropenia) were observed in almost all the cases. It was usually slightly macrocytic and orthochromic or hypochromic in type. Though the relative proportion of lymphocytes was high their absolute number was not increased. Some cases showed slight or moderate eosinophilia but this might be due to helminthic (*ascaris* and hookworm) infection which was common. The sedimentation rate was always increased. Investigation for any hæmorrhagic state usually showed some lowering

of platelet with prolongation of bleeding time. The prothrombin time and coagulation time (Lee and White) were usually normal. Sixteen cases tested for Rh type were all Rh positive : Rh₂-1, Rh₁-14, Rh₂-1. Mothers' blood could be obtained only in three cases and they all showed the same Rh type as that of the patient. Rh incompatibility does not, therefore, appear to be responsible for the pathological changes as suggested by Tidy (1952) for Banti's disease.

2. *Adrenaline test.*—This test was performed in 26 cases to estimate the degree of sequestration of the formed elements of blood in the spleen following the technique of Dean and Wright (1946). 0.5 c.c. of 1 : 1,000 adrenaline (for adult patient) was injected subcutaneously after an initial estimation of blood count from the venous blood. Thereafter, serial samples of venous blood were taken for two hours and the circulatory response (changes in blood pressure and pulse rate) and splenic contraction were also noted.

The circulatory response could not be definitely correlated to hæmatological response or splenic contraction. The splenic contraction, however, synchronized quite well to the hæmatological response. The maximum splenic contraction varying from $\frac{3}{4}$ to 4" (average 1.9") occurred at 5 to 30 minutes and the maximum hæmatological response at 10 to 30 minutes. In case of huge splenomegaly with extensive fibrosis, contraction was poor (though a favourable condition for sequestration and phagocytosis). The maximum rise of Hb., r.b.c., w.b.c. and cell volume varied from 0.29 g. to 5.8 g. per cent, 140,000 to 244,000 per c.mm. 0 to 5,800 per c.mm. and 3 to 14 per cent with a mean of 2.66 g. per cent 1,170,000 per c.mm. 1,887 per c.mm. and 7.8 per cent, respectively. Computed as percentage rise above the base level these figures come to hæmoglobin 5 to 63 per cent (mean 26.5 per cent), r.b.c. 4 to 78 per cent (mean 40.9 per cent), w.b.c. 0 to 220 per cent (mean 48.5 per cent) and cell volume 10 to 52 per cent (mean 27.2 per cent). The maximum rise of neutrophils, lymphocytes, monocytes and eosinophils varied from 0 to 2,467, 18 to 2,700 0 to 254 and 0 to 720 with a mean rise of 783, 1,007, 73 and 223 which expressed as percentage above the base level amounted to an average rise of 41.6 per cent, 78.2, 103.1 and 106.6 per cent, respectively. The test was not pursued long enough for the lymphopenic and eosinopenic effect of adrenaline but variable reduction of lymphocytes and eosinophils below the initial level was seen in 19 and 15 cases, respectively, out of 25 where the test was carried out till two hours.

The results obtained with the adrenaline test indicated mobilization of reserves, chiefly from the spleen. The increase of red cells and cell volume in the present series is very significant and much higher than one can expect in a normal case. The average rise of r.b.c., 1.17 million/c.mm. or 40.9 per cent (0.315 million/c.mm. or 6.6 per cent in normal cases) (Chatterjee *et al.*, 1953) above the base level synchronizing with the contraction of spleen indicates their sequestration in the organ where they are very liable to phagocytosis by the hypertrophied reticulo-endothelial cells. This explains the depletion of red cells in the peripheral blood. Treatment with hæmatinics usually stimulates hæmopoiesis and corrects the anæmia for a time but it recurs soon after discontinuation of treatment so long as hyperactive spleen remains. The rise of w.b.c. is probably contributed both by spleen and bone-marrow after adrenaline injection. The rise observed here is not higher than those of normal cases. Leucopenia is,

however, almost a constant finding in tropical splenomegaly and the spleen seems to have a share in its production. This point can be properly assessed from a systematic follow-up of splenectomized cases and by studying the bone-marrow. The observation so far made shows a rise to normal level of the leucocytes after splenectomy. The bone-marrow studied in a few cases was hyperplastic.

3. *Hepatic function and biopsy.*—The bilirubin content was above 1 mg. per cent in eight cases. The total serum protein was usually within the normal range but in most of the cases albumin was reduced, globulin raised and A : G ratio lowered. The thymol-turbidity test performed in 31 cases gave negative reaction (0 to 4 units) in 13 and positive reaction (4.7 to 14 units) in 18. The prothrombin time estimated in 28 cases was 14 to 16.5 seconds in 11 and 17 to 22 seconds in 17 with a mean value of 17.5 seconds. The normal prothrombin time was 14 to 16 seconds with the method used.

Specimens of liver tissue obtained by aspiration biopsy was examined in 16 cases. Both hæmatoxylin-eosin and silver impregnation (for reticulin) preparations were examined. Eight out of 15 cases of uncomplicated 'splenomegaly' did not show any abnormal hepatic structure. The other seven showed a few foci of degeneration of hepatic cells with lymphocytic infiltration and slight patchy increase (usually at the degenerated area) of reticulin in some of them.

4. *Spleno-portal venography.*—This technique was adopted to visualize the splenic and portal venis by injecting a radio-opaque substance directly into the spleen. The venography has been done in twenty cases of tropical splenomegaly and two of malaria with slight enlargement of spleen (control). The test was performed (after a preliminary test dose) in the first case with 70 per cent diodone which was replaced later by 38.5 per cent Iodoxyl with equally good results. Some side reactions, such as nausea, vomiting, slowing of the pulse, dilatation of the pupils and pain in the splenic area were often seen after the injection. There was no fall of blood pressure, occasionally a rise of pressure was observed following the injection of Iodoxyl. One patient had signs of collapse but responded promptly to the usual measures. A slight rise of temperature and tenderness at the site of injection for a day or two was commonly seen.

Our preliminary observation in monkeys where the dye was injected after laparotomy into the spleen showed that within a short time after injection of the dye, the splenic substance at the site became whitish and necrotic with roughening of the surface. Histological section showed poorly staining nucleus and cytoplasm at the area. Observation in a splenectomized patient also showed necrosis at the site of previous injection of the dye. A small amount of blood-stained fluid may also collect in the peritoneal cavity (Babuson *et al.*, 1953).

A great concentration of the dye was seen in the spleen at the site of the injection and from there it could be traced to the venules in the organ and then through the splenic and portal veins to the liver. In the two malaria cases with slight splenic enlargement only this course was followed and there was no abnormality, such as excessive dilatation (diameters were measured in x-ray films), tortuosity, visualization of tributaries (apart from intrahepatic branches), etc. In the 'splenomegaly' group the diameters were increased in most of the cases. While it had

a relation with the size of the spleen suggesting functional dilatation, this was not invariable. The intrahepatic branches were visualized in all except four where the non-visualization was probably due to dilution and poor concentration of the dye or other technical difficulties since serial films could not be taken. In all except two, no stream-lining was seen and the dye was to all parts of liver, the right being rather more prominent contrary to expectation. In two, however, a left stream lining was seen in the portal vein though in the liver intrahepatic branches of both sides were opacified.

Two patients with spleen enlargement of 3" and 5" showed narrowed splenic veins (0.6 cm. and 0.7 cm. diameter) which, however, expanded to 1.7 cm. before joining portal vein (1.8 cm. and 2.2 cm. diameter). The narrowing is suggestive of thrombosis with portal canalization. The dilatation and tortuosity in some cases might be due to increased portal tension which was also suggested by the visualization of smaller tributaries (the dye having entered them against the usual direction of blood flow), such as left gastric, gastro-epiploic, pancreatico-duodenal, etc., in eight cases.

5. *Splenectomy*.—Splenectomy was performed in six cases after improvement of general health with hæmatinics, blood transfusion, etc. One of them was associated with full-term pregnancy. She was operated three weeks after a normal confinement. One patient died after the operation, while all others showed much improvement. The anæmia disappeared and the total leucocyte count returned to almost normal figure though slight neutropenia and relative lymphocytosis persisted. Two patients had *vivax* paroxysm after splenectomy possibly due to lowering of resistance to malarial infection. Histological examination of the spleen showed the usual picture of tropical splenomegaly, viz. dilated sinusoids, atrophy of lymph follicles, fibrosis, reticulo-endothelial hyperplasia and phagocytosis of red cells. There was no peri-arteriolar hæmorrhages and fibrosis as seen in Banti's disease. Perisplenitis was common. Attempt was made to study the vascular pattern of the spleen by injecting radio-opaque material into the vessels and taking skiagrams of the organ. This showed dilated vessels and sinusoids compared to normal spleen obtained from cases of accidental deaths.

6. *Field studies*.—Field studies commenced last year to observe the splenic enlargement in a malarious village under natural conditions could not be continued for lack of co-operation. If possible the work will be commenced again on another group of villagers.

7. *Experimental study in animals*.—It was found in the course of experiments with *P. berghei* in rats that some of the animals after the acute infective phase became immunized. Such rats after an interval of two to three months were still found to have a spleen volume and weight about double the normal though the blood was free from parasites. This line of work will be pursued to see if malarial infection may leave a residual splenomegaly that persists and if this leads to any changes in the blood picture as seen in 'tropical splenomegaly'.

Comment.—Histopathological changes of the spleen are indicative of its hyperactivity and in the light of these changes as well as the presence of bone-marrow hyperplasia and results of adrenaline test it appears that hypersplenism is an important factor in producing the blood picture. The splenic hypertrophy and hyperfunction brought about by malarial attacks

probably tends to persist and after repeated attacks leads to the hypersplenic syndrome. Splenic fibrosis, dilatation of the spleno-portal veins and changes in the liver in some cases possibly occur subsequently.

EPIDEMIC DROPSY.

The demonstration of the toxic action of argemone oil in animals and the production of an epidemic-dropsy-like condition in monkeys have confirmed its ætiological rôle in the disease. Now the problem is to identify the toxic principle or principles in the oil other than sanguinarine which constitutes only five per cent of the total alkaloids, to work out their respective toxic action in the animal body with a view to trace out the exact toxic substances and, if possible, to find out a suitable antidote.

I. Isolation of total alkaloids from argemone seeds.

It appeared that to commence with, the total alkaloid should be separated and their toxic properties studied before proceeding to the identification, separation and study of the constituent fractions. Work was, therefore, started to obtain an adequate supply of the alkaloids from argemone oil, but the yield was poor, so other methods were explored.

(a) *From argemone oil*.—The method so long used for their isolation from the oil was found to be unsatisfactory for sufficient amount of the alkaloids necessary for experimental work. The cause was attributed to the fact that these alkaloids are only sparingly soluble in oil, and the major portion of the alkaloids of the seeds of *Argemone maxicana* remains behind in the cakes after pressing out the oil.

(b) *From argemone oil cakes*.—Attempt was made to extract the alkaloids from the cake instead with petroleum ether in soxlet. On partially concentrating extract by distillation, dry HCl gas was passed through the liquid when an orange-coloured precipitate (the alkaloid) was found to separate. The yield of the total alkaloid from the cakes was found to be very satisfactory.

(c) *From argemone seeds*.—Next an attempt was made to see if total alkaloids could also be obtained directly from the argemone seeds. The following method was devised for the purpose :—

The seeds were powdered in a metal pestle and mortar, and the paste extracted in a soxlet using a mixture of benzene and petroleum ether (60° F. to 80° F.). From this extract the total alkaloid was isolated as before. Adequate amount (larger than from cakes) could be obtained by this process. The chief advantages of this method over the usual one from the pressed-out oil are :—

(1) Mill-owners invariably refuse to undertake to press argemone seeds. Hence, the oil had to be prepared in the department using a laboratory-model hydraulic press but the yield was only 16 to 18 per cent even after using powdered seeds and pressing out the cakes repeatedly involving an unusual amount of manual labour.

(2) The total alkaloids being relatively insoluble in fatty oils are considerably lost in the process.

(3) The yield of alkaloids is much higher by this method than by (a) and (b) and, therefore, there is greater chance of tracing out the toxic alkaloids which may be present in traces only in the oil.

Using this method 20 g. of total alkaloids has been prepared from about 14 pounds of seeds. Two fractions of total alkaloids were obtained as hydrochloride, viz. (i) soluble and (ii) insoluble in water. The former has been found to contain sanguinarine hydrochloride possibly along with other water-soluble alkaloids as hydrochloride that may be present in the seeds.

II. *Experimental work.*

Studies with the aqueous solution of total alkaloid as hydrochloride on experimental animals have recently been started to find out its toxicity in rats, mice and rabbit.

1. *Acute toxicity.*

(a) *On rat.*—Twelve rats were at first injected either intramuscularly or intraperitoneally with 2 mg. to 5 mg. alkaloid per 100 g. of the body-weight. There was local swelling after intramuscular injection but none died probably due to non-absorption of the toxin. Following intraperitoneal injection there was one death with 2 mg./100 g. and two deaths with 5 mg./100 g. out of three in each group. The surviving animal in the latter group also died after 11 days with septic peritonitis.

(b) *On mice.*—The lethal dose was estimated in mice. When 4 mg. or more per 100 g. body-weight were injected intraperitoneally all the animals died but with a dose of 3 mg./100 g. there was a mortality of 12 animals out of 16 (i.e. 75 per cent mortality). There was no reduction in toxicity (lethal effect) when the solution was heated for an hour at 100° C.

Histological examination of the post-mortem material of the mice did not show generalized congestion as after chronic argemone poisoning. There was, however, intense engorgement of lung capillaries with hæmorrhage areas. The kidney was also congested at places.

(c) *On rabbit.*—The toxin was also administered intravenously to a rabbit weighing 1 kg. Out of 20 mg. administered at the first injection about 8 mg. leaked into the subcutaneous tissue where a hæmorrhagic swelling appeared. The animal looked ill and lost some weight after a week when a second intravenous injection of 10 mg. was given. A post mortem was done following the death of the animal next day. The site of subcutaneous injection showed hæmorrhagic necrosis and detachment of parts of the ear. Straw-coloured fluid in fair amount was seen in the pericardial and pleural cavities and a big fibrin clot had formed in the right pleural fluid. Both the lungs showed hæmorrhagic spots at the margins. Histological sections revealed congestion, hæmorrhagic œdema and cellular infiltration (mainly interstitial) in lungs, exudation into Bowman's capsule and some congestion of kidney and fatty infiltration of the liver with foci of degeneration and replacement by cellular infiltration. In short the changes were dilatation and increased permeability of capillaries and degeneration of hepatic cells.

2. *Chronic toxicity*.—Solution of argemone alkaloid sterilized by boiling (which did not reduce toxicity) was used next in four mice with a dose of 1 mg./100 g. intraperitoneally daily. These caused a progressive loss of weight to 66 g. from the initial 79 g. (total weight of four mice) after a week but no other apparent change. The dosage was then increased to 2 mg./100 g. which caused death of the animals in about another week. This administered with the milk in a dose of about 0.5 mg. per mouse increased later to double the amount. These quantities are only approximate as all the animals were served together with the alkaloids mixed with milk, part of which again was often rejected. The feeding was continued for eight weeks during which four animals died (after two, five, seven and eight weeks). Other symptoms observed were progressive loss of weight and areas of alopecia which appeared after about six weeks. Argemone feeding was stopped for the single surviving animal and it was reverted to usual diet. This animal died after another two weeks.

Histological examination of the tissues of these animals showed : Heart—patchy degeneration of muscle fibres with areas of slight congestion; lungs—marked engorgement of capillaries with exudation or r.b.c. into alveolar spaces at places ; liver—dilatation of sinusoids with fatty infiltration ; kidney—marked congestion and lymphocytic infiltration. Suprarenal gland congestion ; skin—dilated vessels and some cellular infiltration in dermis.

3. *Effect on capillary permeability*.—Menkin's method was used for demonstration of permeability of skin capillaries of rabbits, directly.

(a) 0.1 c.c. solution containing 5 μ g. to 10 μ g. of the alkaloid was injected intracutaneously on the shaved abdomen.

(b) 0.1 c.c. of histamine solution 10 μ g. to 20 μ g. about the same time as a control test.

(c) Then trypan blue was given Intravenously.

Results.—The alkaloid produced an intense blue area (1 to $\frac{1}{2}$ " $\frac{3}{4}$ " diameter) in $\frac{1}{2}$ an hour, indicating local increase of capillary permeability and extravasation into the skin of the intravenously administered colloidal dye. Histamine produced a similar but more diffuse change and it appeared more quickly with a pale area in the centre. A further difference between the effect of argemone alkaloids and histamine on the capillaries was obvious from the fact, that increased penetration of dye was observed even if the dye was administered intravenously up to 24 hours following the local injection of argemone alkaloids whereas in the case of histamine this interval did not exceed two hours.

4. *The direct observation of the toxic effect of argemone alkaloids in the rabbits ear chamber*.—The technique for the direct observation of capillaries *in vivo* in the rabbit's ear has been developed using the modified Sandison Clark chamber described by Ebert *et al.* (1939). It is proposed to inject the alkaloid intravenously and observe changes in blood vessels (i.e. telangiectasis, hæmorrhage, formation of new vessels) that may occur.

III. *Effect of drugs in counteracting the toxic effect of argemone oil and alkaloids.*

Studies on the effect of drugs in counteracting the toxic effect of (1) newly isolated alkaloids, and (2) argemone oil are also in progress. The following observations have so far been made :—

1. *Argemone alkaloids* :—

(a) *Protection against the lethal effect of argemone alkaloids*.—British anti-Lewisite, phenergan, ephedrine and cortisone were tested for this purpose using dose of 3 mg./100 g. of argemone alkaloids that normally gave 75 per cent mortality. The drugs were administered intramuscularly 40 to 50 minutes before the intraperitoneal injection of the alkaloids. Since a heavy dose was used, the drugs were also administered alone in the same dosage to other groups of eight mice each. There was no adverse effect except in one mouse which had a transient convulsion following the injection of BAL. The results of treatment are shown in Table III :—

TABLE III.

Group	Number of mice	Average weight per mouse, g.	Drug used	Average dosage per kg.	Number of deaths	Remarks
1	8	20	<i>Nil</i>	...	6 (75 per cent)	The deaths occurred within 48 hours.
2	8	16.4	Phenergan	76 mg.	7 (87 per cent)	No appreciable difference from control (group 1)
3	8	14.6	Ephedrine	84 mg.	6 (75 per cent)	
4	8	17.75	BAL	35 mg.	4 (50 per cent)	
5	8	19.5	Cortisone	64 mg.	8 (100 per cent)	No death within 48 hours.
6	9	19.1	BAL (for 3 days)	1st day 36 mg. 2nd and 3rd days 18 mg.	2 (22 per cent)	All died within 24 hours.
7	8	19	BAL (for 3 days) & cortisone (injection)	1st day 32 mg. 2nd & 3rd days 16 mg.	8 (100 per cent)	One died within 24 hours and another in 96 hours.
						Five died within 48 hours.

There was apparently no protection against the lethal effect of the toxin with phenergan and ephedrine (groups 2 and 3). BAL used in a single dose (group 4) afforded only slight protection (50 per cent mortality) but there was no death in the first 48 hours. The chief result, therefore, was that death was delayed. When a higher dose of argemone alkaloids, viz. 6 mg./100 per g. was used in 14 mice previously treated with BAL (34 mg./kg.) and 15 control mice (given argemone alkaloids alone) there were two deaths in the treated and ten deaths in the control group after four hours, while all the animals died within 48 hours in the treated and 24 hours in the control groups. So death was only delayed here by BAL without any protection. From these results it was thought that more satisfactory

response might be obtained if the injections of BAL were repeated with an argemone dosage of 3 mg./100 g. This proved to be true as is shown in group 6 of Table III with a mortality of 22 per cent. When the same 3-day regimen of BAL treatment was, however, given along with a single injection of cortisone the protective action of the former was completely annulled and all the eight animals died (group 7). Cortisone alone caused rapid death of all the animals within 24 hours following argemone injection. Cortisone was tried because of its reported protective action on capillary permeability (Scieftes *et al.*, 1949; Benditt *et al.*, 1950) but the toxic effect of argemone alkaloids appeared to be enhanced by cortisone. Here, possibly another action observed with ACTH, viz. a lowering of the sulphydryl level of blood (Goldzicher *et al.*, 1953) predominated especially with the high dosage used. It appears possible that the sulphydryl level of the body already diminished by cortisone was more quickly and completely inactivated by the argemone alkaloid and BAL, even in liberal doses, could not afford sufficient protection.

(b) *Protection against increased capillary permeability produced in the rabbit's skin.*—The trypan-bluet technique for the demonstration of increased capillary permeability following intracutaneous administration of very low dilution (5 μ g. to 10 μ g. in 0.1 c.c.) of argemone alkaloid was also used to see if previous treatment with some drug could prevent the increase of permeability. It may be mentioned that the increase of permeability produced by histamine (10 μ g. to 20 μ g.) could be suppressed by pre-treatment with phenergan.

Phenergan (7 mg./kg. intravenously, given $\frac{1}{2}$ hour before) did not, however, counteract the action of the argemone alkaloids nor did pre-treatment with British anti-Lewisite (16 mg./kg. intramuscularly for four days), although the latter gave some protection from the lethal effects of the alkaloids (*see above*). Rutin (20 mg./kg. for three days by mouth) and ephedrine (30 mg./kg. intravenously, 20 minutes before the test) were also ineffective.

Pre-treatment with cortisone (10 mg./kg. intramuscularly for several days) did not also protect the capillaries against the action of argemone alkaloids to a significant extent, as judged from the amount of trypan blue which entered the skin at the site of local injection of the alkaloids.

2. *Protection against chronic argemone oil poisoning.*—Twenty-eight rats (average weight 80 g. each) were divided into seven groups of equal number and five groups fed daily with 1 c.c. of 30 per cent argemone oil in mustard oil for each rat by a special metal catheter attached to a syringe. Four groups were treated with drugs, while one served as control. Two more control groups (sixth and seventh) were introduced one having no feeds or treatment and the other fed with pure mustard oil. The four treated groups received BAL (3.4 mg./kg.), phenergan (2.6 mg./kg.), ephedrine (10 mg./kg.) and vitamins (A-4,000 units, D-400 units, E-0.8 mg., C-20 mg., thiamine-24 mg., riboflavin 0.4 mg., niacin 8 mg., pyridoxin 1.2 mg., and rutin 8 mg./kg.). All the drugs except rutin (given orally) were administered daily by intraperitoneal injection.

The skins of all the argemone-fed animals showed some changes: hair lost their glossiness and were rough and sparse. There was no significant difference in weight gain in the different groups including controls excepts that in the ephedrine group the gain in weight was slightly higher. The animals were sacrificed after a month and sections of heart, liver and kidney stained by hæmatoxylin and eosin and Pickworths technique examined

for the assessment of results. The vascular changes in the treated group did not show any significant difference as compared to the control group fed with 30 per cent argemone oil alone apart from one animal in the ephedrine group where the changes were slight. This, like similar change in one animal of phenergan group seen last year, is not probably significant.

It may be concluded, therefore, that treatment with BAL, phenergan, ephedrine, rutin and vitamins cannot protect the animals from the vascular effects of chronic argemone poisoning.

The failure of protective action of BAL from the increased capillary permeability produced by argemone alkaloids and from the vascular effect of chronic argemone oil poisoning may explain why BAL though affording some protection to animals from the lethal effect of argemone alkaloid, could not alter the clinical course of epidemic dropsy in two cases (Chaudhuri and Chakravarty, 1950).

IV. *Toxicity of white oil.*

(i) *Toxic action after oral administration.*—Last year it was observed that the toxicity of white oil in monkeys was variable in different batches and the more toxic samples exerted a harmful effect even when administered in a dilution of 10 to 25 per cent for long periods. This year we found two other samples of white oil toxic to mice, rats and rabbits when administered undiluted or in 30 per cent concentration in mustard oil. The toxicity was demonstrated by the failure to put on weight, lethal effect in two to three weeks time in some of the animals and tissue changes of the same nature as described before. In case of rats and mice the hair lost glossiness and became rough, coarse and sparse. This, however, was partly a non-specific reaction as administration of liquid paraffin also produced somewhat similar changes and in both it tended to disappear on withholding oil feeding for a few days. Histological sections, however, showed more definite changes in the white oil treated animals (*see below*). The blood count and biochemical changes were also observed in rabbits. There was an increase of urea, N.P.N. and total lipids of blood but no hæmatological changes were seen.

In order to see if liquid paraffin itself may have any harmful effect, two groups of three rats each were fed with 1 c.c. to 2 c.c. of liquid paraffin (B.P.) and white oil daily for about six weeks with some intermissions at the end of which the animals were sacrificed. The liquid-paraffin group gained weight normally but developed the skin changes mentioned above possibly due to lack of absorption of vitamins. Animals in the white-oil group showed retarded weight gain and in addition to usual skin changes, cracks, fissures and epilation of ventral surface. Histological examination showed hyperkeratinization and increased vascularity of dermis in white-oil group and no significant change in the paraffin group. Vascular engorgement with hæmorrhages at places was seen in the heart, lungs, liver, kidney and suprarenal glands of the white-oil group. The liquid-paraffin group showed no significant changes apart from some congestion of the kidney.

(ii) *Absorption of white oil from skin surface.*—Three shaved animals were dipped once daily in white oil up to the neck for three consecutive days. The skin surface became red and the animals looked ill. Two

animals died on the fourth day and one on the fifth day. On naked-eye examination the organs appeared very congested and histological sections revealed : Skin—hyperkeratinization with parakeratosis and atrophy of prickle-cell layer. Heart—congestion and areas of hæmorrhage between muscle fibres. Lungs—marked congestion with hæmorrhage into alveoli at places. Liver—dilatation of sinusoids and central veins. Kidney—patchy areas of congestion and hæmorrhage. Three other rats were also similarly treated with liquid paraffin (B.P.) for seven days without any harmful effect whatsoever. This experiment suggests that white oil or its toxic principle is absorbed through the skin.

(iii) *Attempt at ' purification of white oil.*—The experiments with ' pure ' and ' impure ' white oil commenced last year has been concluded. The oil feeding was continued for two months during which two animals died in either group of three after nearly equal intervals, the weight changes were also almost parallel and histological sections of tissues of both the groups of animals showed the usual vascular changes observed for white oil poisoning. It may be concluded, therefore, that either the oil itself is toxic or the impurities responsible for the toxic action were not totally removed by the method of purification employed.

CHOLERA

1. *Plasma sodium, potassium and chloride changes in cholera and their significance in prognosis and treatment.*

Some electrolytic changes were studied in 18 cholera cases. Fifty-one observations were made on these 18 patients admitted to the Cholera Ward of Nilratan Sarkar Medical College Hospital. The estimation of sodium and potassium was done by Flame-photometer. The cases can be grouped as follows :—

(A) In six seriously ill cases estimations were done on admission before any treatment was given and in the five surviving cases estimations were repeated immediately after the first infusion of the saline (usually 600 c.c. of 1·2 per cent NaCl and 1,200 c.c. of 0·9 per cent NaCl), four hours later and on the 2nd, 3rd 4th and 5th days. The average figures for plasma sodium, potassium and chloride were as follows. On an average they had about 3·5 L of saline in first 48 hours and no potassium salt :—

m Eq/L	Before treatment	Immediately after saline	4 hours after saline	Days			
				2nd.	3rd.	4th.	5th.
Na+	148	146	145	145	142	142	145
K +	6·6	5·6	4·6	4·6	4·3	4·2	3·3
Cl—	98·0	97	96	98	96	98	97

Plasma sodium and chloride did not show much variation throughout the whole course. Potassium, however, showed high figure which declined

almost immediately after the first infusion and gradually came down to normal and in two cases became even sub normal on the fifth day.

(B) Eight cases of cholera, developing uræmia and/or pulmonary œdema during the course of their treatment in the hospital, showed very high urea concentration of the blood. Plasma sodium was high (160 and 165 m Eq/L) in two of them which developed pulmonary œdema and who had a large quantity of saline infusion (10 and 14 pints) within a short period of time to prevent repeated circulatory collapse. Except those two cases, the rest had very high plasma-potassium concentration (10.0, 5.8, 7.3, 7.5, 9.0, and 6.5 m Eq/L).

(C) In three cases of uræmia, which terminated favourably, plasma-potassium concentration which was very high during uræmic stage, gradually came down to normal along with the blood-urea concentration; in one case it actually came down to even a sub normal level. In one case of bacillary dysentery with severe dehydration, the changes observed were similar to those in cholera cases.

It appears from the above study that in cholera there is little change in the initial plasma-sodium and chloride level, indicating that the loss of fluid in cholera is possibly isotonic in nature. But initial plasma-potassium level is the usual finding as reported also by Saha and Das (1951). It may even come down to subnormal level with treatment and cause prolongation of convalescence, and if there is free flow of urine, potassium salt may be exhibited with profit to hasten convalescence.

If there is too much saline infusion within a short period of time to prevent repeated circulatory collapse encountered in some obstinate cases there is a positive danger of inducing acute pulmonary œdema. In uræmia cases invariably there is very high rise of plasma potassium along with nitrogenous retention. Such dangerously high level of plasma potassium is probably an important contributory factor in their fatal termination and every effort should be made to handle this high potassium concentration during their treatment. Treatment as suggested by Bull *et al.*, (1949) is indicated. In cases where uræmia is terminated favourably plasma potassium declined rapidly and so also the urea concentration in the blood.

II. *Further observation on intravenous chloromycetin in cholera.*

In continuation of the work done last year (Chaudhuri *et al.*, 1952) a group of 50 cases of cholera was treated with intravenous chloromycetin with another 50 alternate cases serving as control. In 12 cases the dosage was 0.5 g. on admission and four injections of 0.25 g. each six hourly and in 38 cases slightly bigger dose was given within a shorter period, viz. three injections of 0.5 g. each every four hours coming from the time of admission. This was well tolerated and the results were same as in the other group.

The overall clinical impression was that the course of the disease in the treated and control group did not differ much. Total quantity of saline required in these two groups of cases on an average was same; i.e. 7.2 pints and 7.3 pints for the treated and control group for each patient. Stool, however, became formed little earlier in the treated group. The most striking result was on the excretion of *V. cholera* in the faeces. The results are given in Table IV :—

TABLE IV.

Showing results of chloromycetin injection on the excretion V. cholerae in stool.

Day of disease	CHLOROMYCETIN GROUP			CONTROL GROUP		
	Vibrio positive			Vibrio positive		
	Total examin- ed	Number	Per cent	Total examin- ed	Number	Per cent
1st (before treat- ment) ...	50	38	76	50	37	74
2nd day ...	35	17	51	32	24	75
3rd day ...	34	4	12	32	8	25
4th day ...	34	Nil	Nil	32	14	44
5th day ...	34	Nil	Nil	32	8	25
6th day ...	34	Nil	Nil	32	5	14
7th day ...	34	Nil	Nil	31	3	10

Intravenous medication by passing the gastro-intestinal tracts has definite advantage over the oral route in the treatment of cholera. But the net result was almost the same as the oral chloromycetin administration (Chaudhuri *et al.*, 1950). The actual therapeutic value of this drug in cholera is very limited. Only beneficial effect noted was that the stool was formed slightly earlier than in the control group. This did not, however, have any effect on the actual quantity of fluid that had to be infused. Rapid disappearance of *V. cholerae* from the stool was a constant finding and this fact, however, may be important from the public health point of view.

III. Treatment of uræmia in cholera with vitamin C and anti-histaminics.

In a recent note Chatterjee (1952) claimed very good result in the treatment of uræmia in cholera with vitamin C and antihistaminics. Details of his work and any explanation for this régime of treatment were lacking, but in view of the fact that out of 700 cases admitted under him, not a single case died of uræmia after the introduction of this régime, it was decided to evaluate the effect of these drugs.

Total number of 78 cases of cholera was studied. They were admitted to Nilratan Sircar Medical College Hospital and had the usual saline treatment for cholera. The cases can be divided into the following groups :—

Group A.—In 25 cases, vitamin C (Redoxin) 500 mg. I.V. was given on admission along with the first saline infusion. If anuria or oliguria persisted, another injection of 500 mg. was given after 12 hours. If the patient failed to pass urine even after 24 hours, antihistaminic (antistin) 2 c.c. was injected I.M. in one of the loins as advocated by Chatterjee. Subsequently, vitamin C and antihistaminic were repeated every 12 hours till the patient passed urine freely or died. Another alternate twenty-five cases

served as a control without these injections. Details of clinical features and the day-to-day progress of these cases were noted. They were comparable groups clinically and on cultural examination of stools as well. The result showed that in the treated group, three patients died of acute circulatory collapse within 24 hours of the admission and another case developing uræmia died on the fourth day. He had five injections of vitamin C and three injections of antistatin-and blood-urea N before his death was 88 mg. per cent. In the control group of cases there were two deaths from acute circulatory collapse.

Group B.—In this group twelve cases were included. After first infusion of saline, if the oliguria or anuria persisted for more than 2-3 hours, injections of vitamin C and antihistaminic were given and repeated every 12 hours till the patient passed urine freely or expired.

Of the 12 cases in this group duration of illness prior to admission was between 8 to 24 hours (on an average of 12 hours). All were seriously ill patients with history of suppression of urine varying from 2 to 20 hours. Three cases expired—two from circulatory collapse within 20 hours after admission and the other case developed uræmia and died on seventh day. He had a total of nine injections of vitamin C and antihistaminic and blood-urea N went up to 80 mg. per cent. Rest of the cases had uneventful recovery.

Group C.—This group comprised of 19 cases. While being treated for cholera in the hospital they developed uræmia, and the usual practice for the treatment of such uræmia cases was followed, such as maintenance of fluid balance, no salt, sodium bicarbonate and glucose injection, oxygen inhalation, etc. Besides such measures, eight of these patients received 500 mg. of vitamin C (I.V.) and 2 c.c. of antistatin (I.M.) every 12 hourly, till they recovered or died. Another eight cases without these two drugs served as control.

All these cases showed very high nitrogenous retention and mortality was very high in both these groups. In the treated group five died out of eight and in the control group six died out of eight.

Out of these three groups of cases, results in groups A and B indicate that vitamin C and antihistaminic could not prevent the complication of uræmia in cholera cases and when developed during this régime of treatment they could not help in the recovery. In group C the results show that when uræmia was well developed, this régime of treatment had no curative effect either.

9. Liver diseases research unit under Dr. P. N. Wahi at the S. N. Medical College, Agra.

SYNOPSIS.

Out of a total of one hundred and nine cases of liver diseases available, ninety-two cases were studied for any possible relationship between structural and functional alterations in the liver. The results of hepatic tests have been classified into four grades of abnormality and tabulated. The average of results of hepatic tests in different degrees of liver-cell

damage in 92 cases has been determined. It has been found from consideration of averages alone that albumin and globulin values, A: G ratio, thymol flocculation, colloidal gold, cephalin flocculation and serum bilirubin, show a proportionate degree of abnormality in different grades of liver damage. It was further found that in grade one *plus* liver-cell damage, thymol turbidity and thymol flocculation might be within normal limits. Analysing the results for portal cirrhosis, infectious hepatitis, chronic malaria and congestive heart failure, it was found that A: G ratio, cephalin flocculation and albumin values were the only tests, the variations of which were correlated with grades of liver damage. Total proteins, thymol turbidity and sedimentation rate were the most unrelated. The best correlated liver function tests in the disease mentioned above have been worked out. It has been observed that the grade three of liver damage has at least six tests abnormal. The nature of these tests, however, varied slightly from case to case. Correlating the clinical findings, it was observed that with advancing liver damage, an increasing percentage of cases had ascites and a decreasing number showed clinical hepatomegaly. Jaundice bore no relationship. A composite correlative study of hepatic injury in rats by acute carbon-tetrachloride poisoning has been started. This comprises histologic, histochemical, and chromatographic studies for amino acid and in liver homogenates. The experiments are still under way.

Qualitative and quantitative chromatographic variation of the amino acids in the urine of carbon-tetrachloride-poisoned rats has been studied. Ascending double-dimensional paper-partition chromatographic studies of standard amino acids have been completed and R_f values of 31 amino acids so studied have been reported. Standard chromatogram showing spots and R_f values of amino acids as they occurred in the solvent, phenol and acidified n-butanol has been prepared. The colour of the spots after ninhydrin reduction has been given. Chromatographic studies qualitative and quantitative, using ascending double-dimensional and circular-paper techniques, respectively) of free amino acids in liver and urine of normal rats has been undertaken. Results till date have been reported. All India Registry of hepatic pathology has been securely established. One hundred and sixty cases comprising various types of liver disease, complete with clinical history, sections and blocks, have been registered so far.

A. STUDY OF RELATIONSHIP BETWEEN STRUCTURAL AND FUNCTIONAL ALTERATIONS IN THE LIVER.

A composite liver-function study, including a detailed clinical study, a battery of 10 to 13 liver-function tests and a liver biopsy within a week of these liver-function studies, has been done in the Department of Pathology, S. N. Medical College, Agra during the last two years. It was considered useful to analyse the results statistically and to find out whether any significant correlations could be established between the structural changes in the liver, the liver-function tests and clinical findings. With this aim in view, the study was directed into the following channels :—

1. To determine whether a statistical relationship can be demonstrated between the abnormalities in the results of hepatic tests and degree of liver-cell damage.

2. To determine whether a statistical correlation exists between the abnormalities in the results of hepatic tests and the type of liver-cell damage.
3. To determine, which, if any, of the hepatic tests show the best correlation and, therefore, best reflect the type of change in the liver cell.
4. To determine if the number of tests with abnormal results is related to the degree of liver-cell damage.
5. To select the hepatic tests which may be used as screening for the presence of liver-cell damage.

Grading of morphologic changes.—The classification of different types of morphological changes in the liver cells was described in detail in the last year's report, except that the term 'portein deficiency' has been replaced by a non-committal term 'plant cells of Gillman and Gillman'. Each of these changes were graded as : O=absent ; + =Slight ; ++ =moderate ; and +++ =marked. In addition, the diffuse liver-cell damage was divided into four grades : O, 1, 2, 3 and by the following criteria (Hopper *et al.*, *Am. Jour. Clin. Path.*, **19**, p. 710, 1949).

Grade 0 (normal).—The cytoplasmic and nuclear size and appearance is uniform in the same zone. Minor variations in the size of the mid-zonal and perihperal zone nuclei are physiological, the former being slightly larger.

Grade 1.—It indicates a moderate variation in the size of the cells and nuclei in the same zone of the lobule. The cytoplasm may show a loss of regular granulation of glycogen and small, irregularly distributed vacuoles due to a hydropic change.

Grade 2.—This is indicated by an irregularity of the arrangement of the cell in the liver-cell cords with exaggerated changes of grade 1, *plus* one or more of the following conditions :—

- (a) Nuclear alterations, e.g. pyknosis, karyolysis and ballooning of moderate degree.
- (b) Cytoplasmic changes of moderate degree, e.g. coarse meshes of the cytoplasm with vacuolization (plant cells of Gillman and Gillman).
- (c) Presence of eosinophilic or basophilic clumps in the cytoplasm (coagulative necrosis).

Grade 3.—It indicates the presence of the changes described under grade 2 in a majority of cells, specially :—

- (a) Marked variations in the size of nuclei and cells.
- (b) High degree of nuclear abnormalities listed under grade 2.
- (c) Marked acidophilic clumping with presence of many 'Mallory bodies'.
- (d) Presence of anuclear cell fragments of complete necrosis of cells.

Grading of hepatic-function tests results.

The results of the hepatic tests were arbitrarily classified into four grades of abnormality as shown in Table I to facilitate the correlation with liver-cell damage :—

TABLE I.

Showing variation from normal of the values of liver-function tests results according to the grade of abnormality.

Test	GRADE OF ABNORMALITY			
	0	+	++	+++
Total proteins ...	Above 5.98	5.97-5.50	5.49-4.40	Below 4.4
Albumin ...	Above 3.96	3.95-3.20	3.19-2.7	Below 2.7
Globulin ...	Below 2.21	2.22-2.69	2.70-3.10	Above 3.10
A: G ratio ...	Above 1.6	1.6- 1.26	1.25-0.9	Below 0.9
Thymol turbidity ...	Below 3	3-6	7.10	Above 10
Thymol flocculation ...	0 to +	++	+++	++++
Colloidal gold ...	0+	++++	+++	++++
Cephalin cholesterol ...	0 to +	++	+++	++++
Prothrombin conc. ...	85-100	84-70	69-50	Below 50
	per cent	per cent	per cent	per cent
Serum bilirubin ...	Below 0.4	0.41-1.09	1.1-5.0	Above 5.0
Sedimentation rate ...	Below 10 mm. (Wintrobe)	11-20 mm.	21-30 mm.	Above 30 mm.

RESULTS.

The histological grading was done independently by three workers and a fairly close degree of agreement was met with. A total of 109 liver biopsies with function studies were available, but only 92 were adequate enough to be graded. According to the criteria for grading of diffuse liver-cell damage, the cases were distributed as follows :—

Grade 0	...	No case
Grade 1	...	14 cases
Grade 2	...	53 cases
Grade 3	...	25 cases

TABLE II.

Showing the average of results of hepatic tests in different degrees of liver-cell damage.

Hepatic function tests		DEGREE OF LIVER-CELL DAMAGE		
		Grade +	Grade ++	Grade +++
Total proteins	5.50	5.76	5.52
Albumin	2.77	2.58	2.13
Globulin	2.34	2.76	3.027
A: G ratio	1.156	1.054	0.681
Thymol turbidity	2.409	7.59	7.89
Thymol flocculation	0.54+	1.4+	1.8+
Colloidal gold	0.64+	1.71+	2.0+
Cephalin-cholesterol flocculation	1.50+	1.82+	2.27
Prothrombin conc.	43.15	48	37
		per cent	per cent	per cent
Serum bilirubin	1.04 mg.	1.37	3.49
Sedimentation rate	12.76	12.69	14.55

Considering the averages alone, it is apparent that albumin and globulin values, A: G ratio, thymol flocculation, colloidal gold, cephalin-cholesterol flocculation and serum-bilirubin values show a proportionate degree of abnormality in the different grades. Further, it is noticed that in the grade one *plus* liver-cell damage, thymol turbidity and thymol flocculation may yet be within normal limits.

Considering the relationship of liver-cell damage with degree of abnormality of hepatic-function tests, the diagramatic representations of this relationship show a significant correlation in A: G ratio, thymol turbidity, thymol flocculation, colloidal gold, cephalin flocculation and erythrocyte-sedimentation rate in that in higher grades of hepatic damage a higher percentage of cases have a 3-grade functional damage or a liver percentage have 1-damage.

Each one of these tests were then analysed for each of the four groups of diseases studied in the present series, e.g. portal cirrhosis, infectious hepatitis (all stages) chronic malaria and congestive heart failure. Other diseases, e.g. extrabiliary obstruction, kala-azar, carcinoma liver, tuberculosis liver were represented in too small numbers to afford a statistical analysis. Averages were similarly calculated for each one of the function tests in different grades in these diseases. A: G ratio, cephalin flocculation and albumin were about the only tests, the abnormalities of which were correlated with grades of liver damage in all the above diseases. Total proteins, thymol turbidity and erythrocyte-sedimentation rate were again the most unrelated with grades of hepatic damage in all the conditions. The best correlated of function tests in individual diseases were as below :—

TABLE III.

Showing the tests which showed the best correlation in individual diseases.

Diseases	Tests
Portal cirrhosis	A: G ratio, albumin, globulin, cephalin flocculation, thymol flocculation.
Infectious hepatitis	A: G ratio, albumin, globulin, colloidal gold, cephalin flocculation, thymol turbidity, thymol flocculation.
Chronic malaria	A: G ratio, colloidal flocculation, cephalin flocculation.
Congestive failure	A: G ratio, albumin-cepahlin flocculation and prothrombin concentraion.

A study of the relationship of abnormal hepatic tests in different grades of liver-cell damage was also done on the assumption that with increasing damage a larger number of the battery of 13 tests would be abnormal. Only the cases with a minimum of 10 hepatic tests were selected. It was found that all cases of grade 3—had a minimum of six tests abnormal, although a certain, though much smaller percentage of grade 1—cases had as many as nine tests abnormal. The nature of abnormal tests, however, varied.

The above results are conveyed with the obvious reservation that these have been calculated on a percentage basis and not statistically. Attempts are being made to avail of the services of a local statistician and find out the statistical significance of these results. These results will be reported later. The relationship of the type of liver damage with the function tests or with the clinical findings is also under calculation.

Attempt was also made to correlate some of the more important clinical findings with the grades of liver-cell damage. It was noted that with advancing liver damage, an increasing percentage of cases had ascites and a decreasing number had clinical hepatomegaly. Jaundice bore no relationship.

B. COMPOSITE STUDY OF ACUTE CARBON-TETRACHLORIDE HEPATIC INJURY IN RATS.

A composite correlative study of hepatic injury in acute carbon-tetrachloride poisoning in rats has been started. It includes the following studies on the same animal :—

- (a) Histological changes in a liver lobule.
- (b) Histochemical and enzymatic variations in the liver cells.
- (c) Qualitative and quantitative variations of amino acids in liver homogenates. These would be studied by the technique of paper-partition chromatography.
- (d) Amino-acid excretion in urine. This would be a qualitative and quantitative study by paper-partition chromatography. As mentioned earlier, this work has already been started, but remains to be completed, and the results, worked out.

I. *Histological and histochemical study.*

Acute injury with carbon tetrachloride was produced in rats with a single subcutaneous injection of 0.18 c.c. per 100 g. of body-weight. The rats were kept on the stock diet prescribed by the Nutrition Research Laboratories, Coonoor. A batch of rats, with a control rat on the same diet but no injection with Cl_4 , was starved for 24 hours to stabilize the glycogen content of the liver. Sacrifice was done after 2, 4, 6, 12, 24 hours and 2, 3, 4, 5, 6, 8, 10, 12, 14 and 18 days. The study includes alkaline and acid phosphatase, nucleo-proteins, glycogen and fat, besides the conventional H and E stain.

The histochemical work was started with the use of poly-ethylene glycols for sectioning and embedding, as done by the workers at Coonoor. Free samples for the purpose were supplied to us by the National Carbon Co., Calcutta, for experimental use. Different combinations of the molecular weight samples 4,000, 1,540 and 1,000 were used. During May to July, CW 1,540 and 1,000 were impracticable as the latter was liquid at room temperature (110°F. average) and the former also was too soft. The use of these carbo-wax was tempting as they produce a minimal tissue distortion, and exclude several steps of hardening and dehydration. Moreover, if workable, the lower temperatures of these waxes ensure a greater preservation of the heat-labile enzymes. On processing through these water-soluble compounds the same sections can be used for fat stain.

With these attractions, the efforts to devise the best combination of carbo-waxes were continued. CW 4,000, which has an M.P. 58°C. to 60°C. was too brittle unless mixed (8 : 2) with CW 1,000. After July, with the onset of the monsoon, when the temperatures were lower, again these waxes could not be used as they are extremely hygroscopic and on absorption of atmospheric moisture become pasty. With these difficulties, it was concluded that in this part of the country, carbo-waxes cannot be used for more than four to five cooler and dry months of the year. Now, the paraffin wax with M.P. 50°C. to 52°C. is being used with consistently good results.

Acetone is being used as the fixative of choice for enzyme studies and in our experience it is better than alcohol. A 24-hour fixation is employed as, on fixation for a longer time, the tissues are apt to get brittle. For alkaline phosphatase, Gomori's method (*Jour. Lab. & Clin. Med.*, **37**, pp. 526-531, 1951) is used. For acid phosphatase, Gomori's revised technique (*Stain Technol.*, **25**, p. 81 1950) is employed with. Goetsch's modification (*Stain Technol.*, **26**, p. 145, 1951) of using undeparaffinized sections. For pyronin-methyl Green stain for nucleic acids, Taft's modification of Scudder's technique (*Stain Technol.*, **19**, p. 39, 1944) is found more serviceable. Also, Korson's differential stain for nucleic acids using orange green, Methyl green and toluidine blue gave good results. Sudan IV in propylene glycol is being used for fat stain.

The techniques for these stains for histochemical work have now been finalized with modifications to suit our atmospheric conditions. The work is already under way and at this stage it is too premature to give the results.

II. *Qualitative and quantitative variations in the amino-acid content of liver homogenates studied by the technique of paper-partition chromatography.*

The same liver as used for enzyme studies is homogenized in water and the amino-acid content analysed. The technique is described in another section of the report.

III. *Qualitative and quantitative variation of the amino-acid content in the urine of the same rats.*

The individual rats are kept in metabolic cages, and the urine collected is analysed by the same technique.

These studies are being done and no results have yet been arrived at.

C. PAPER-PARTITION CHROMATOGRAPHIC STUDY OF AMINO ACIDS.

Last year the Rf values of 34 amino acids were reported using descending single-dimensional method of Dent (*Biochem. Jour.*, **43**, p. 169, 1948). The study of amino acids by ascending double-dimensional technique of Williams and Kirby (*Science*, **107**, p. 481, 1949) was also started. The work has now been completed and the results are reported below :—

I. *Ascending double-dimensional paper-partition chromatographic studies of standard amino acids.*

Two-dimensional analysis by the ascending method using glass-jars and Whatman No. 1 filter paper, described by Williams and Kirby (*Science*, **107**, p. 481, 1948) was used.

Phenol (Merck or B.D.H.) saturated with a solution of sodium citrate 6.3 per cent and sodium-di hydrogen phosphate 3.7 per cent was used as the first solvent followed by n-butyl alcohol saturated with acetic acid and water in the ratio of 4 : 1 : 5 as the second solvent.

Usually, 0.1 per cent of the amino acid in 75 per cent alcohol was used. Sometimes when the amino acid did not go in solution it was dissolved as its hydrochloride by adding a few drops of hydrochloric acid. If the solution was acidic before application to the paper it was neutralized by the ammonia vapour after its application to the paper so that acidity of the solution may not affect the Rf values. The papers were run in both solvents overnight, both running about 21 to 24 hours. After the phenol run, the papers were dried at room temperature, then turned and run in the n-butyl alcohol following which they were dried again at room temperature for three to four hours before spraying with ninhydrin. After spraying with a solution of 0.25 per cent ninhydrin in n-butynol sturated with water, the papers were again dried at room temperature. The developed spot was marked and the centre of gravity of the spot taken for the calculation of Rf values. The Rf values were calculated separately with respect to phenol and n-butyl alcohol.

The temperature was maintained between 33°C. to 35°C. inside an incubator in which the experiments were conducted. Each chromatogram was run at least ten times for finding stable values. Thirty-one standard amino acids have been studied by this method. The Table IV gives the Rf values of these amino acids with phenol and n-butynol as solvents :—

TABLE IV.

Rf values of the amino acids with respect to phenol and acidified n-butanol separately as studied by double-dimensional analysis using ascending technique.

Amino acids				Rf values	
				Phenol	n-butanol
1.	Aspartic acid	0.05	0.21
2.	Glutamic acid	0.22	0.26
3.	Glycine	0.31	0.20
4.	α-Alanine	0.55	0.30
5.	Hydroxyproline	0.64	0.30
6.	B-Alanine	0.61	0.35
7.	Proline	0.82	0.40
8.	Phenylalanine	0.79	0.56
9.	Leucine	0.81	0.61
10.	Nor-leucine	0.81	0.61
11.	Iso-leucine	0.78	0.70
12.	Valine	0.74D } 0.71H }	0.54D } 0.61H }
13.	Nor-valine	0.75	0.61
14.	Taurine	0.21	0.23
15.	Serine	0.17	0.22

TABLE IV—(contd.)

Amino acids			Rf values	
			Phenol	n-butanol
16.	Histidine	0.54	0.32
17.	Methionine	{ 0.69	0.45
			{ 0.69	0.85
18.	Cysteic acid	0.03	0.11
19.	α -Aminol-butyric acid	0.63	0.42
20.	dl-Citrilline	0.49	0.24
21.	Lysine	{ 0.14	0.14
			{ 0.15	0.39
22.	Arginine	0.19	0.20
23.	Asparagine	{ 0.21	0.19
			{ 0.06	0.20
24.	Threonine	0.32	0.30
25.	Glucosamine	0.11	0.20
26.	Glutamine	0.54	0.12
27.	Orinthine H. Br.	{ 0.16	0.15
			{ 0.06	0.07
28.	Hamocysteine	0.26	0.27
29.	Tryptophan	0.23	0.25
30.	Glutathione	0.38	0.14
31.	3 : 5-Dicodotyrosine	0.80	0.83

Two more amino acids, histamine di-HCl and tyrosine were also studied and their double-dimensional chromatograms run for about a dozen times each but without convincing results. With the former, generally a long streak was always obtained when a solution of 0.1 per cent was used but with dilute solution (up to 0.01 per cent) only a spot in the phenol and no spot when run double-dimensionally was obtained. Tyrosine gave a long streak with Rf values differing in each individual chromatograms. These two amino acids will be studied further and final position ascertained and reported later.

II. *Standard chromatogram showing spots and Rf values of amino acids as they occurred in the solvents, Phenol and acidified n-butanol utilized in the study.*

The map of spots has been prepared from the spots obtained with known amino acids in respect to the two solvents mentioned above. An attempt has been made to represent the shape of the amino-acid spots as observed on a double-dimensional chromatogram, although slight variation in shape and size of the spots was observed in different experiments.

III. *Chromatographic studies of free amino acids in liver and urine of normal rats.*

The free amino acids of normal liver and urine of rats are being studied using the ascending double-dimensional technique described above.

The rats are kept fasting for 24 hours in metabolic cages and their urine collected. They are then sacrificed and their livers taken out. The urine is applied to Whatman No. 1 paper (25 μ l.) by a capillary tubing. The chromatograms are run in phenol followed by n-butanol. To expediate the number of experiments the modified apparatus as mentioned by Datta, Dent and Harris (*Jour. Arch. Biochem.*, **19**, p. 172, 1948). The weighed quantity (0.8 g. to 1.2 g.) of liver is homogenized in boiling water and to the homogenate excess of alcohol is added to precipitate all the proteins. The resulting extract is centrifuged and to the supernatant fluid three times its volume of chloroform is added to dissolve lipoids. The mixture separates in two layers, the upper aqueous alcohol mixture containing amino acids and the lower chloroform alcohol mixture containing lipoids. The upper fluid is pipetted off and concentrated to one-fifth of its volume by boiling. Twenty-five μ l. of the extract is applied to the paper and free amino acids are chromatographed (*see* Table V opposite).

The following free amino acids were present in liver of a normal rat :—

1. Cysteic acid
2. Aspartic acid
3. Glutamic acid
4. Glycine
5. Serine
6. Taurine
7. α -Alanine
8. Histidine
9. Tyrosine
10. Valine
11. Leucine

The last two were, however, not found in all chromatograms. The first nine amino acids were also found in the urine of the normal rats. Numbers 10 and 11 were found in smaller amounts in circular chromatograms only.

Tyrosine was not also found on a double-dimensional chromatogram but was detected by the circular chromatography both in urine and liver tissue free amino acids.

IV. *Quantitative and estimation of free amino acids in liver urine of normal rats from chromatographic studies.*

It was mentioned in last year's report that technique of quantitative estimation from chromatographic studies will be developed with the ultimate purpose of studying (i) progressive amino-acidinurea in hepatic lesions, and (ii) free amino acids and peptides in normal, cirrhotic and cancerous livers. As a preliminary to the above, the quantitative estimation of free amino acids in liver and urine of normal rats has been undertaken. The circular-paper chromatograms run according to the technique of Giri *et. al.* (*Jour. Ind. Ins. Sci.*, **36**, p. 145, 1953) has been found useful and is being used. The work is being still pursued. Till date, results are given in the Table V. Detailed results will be submitted when the experiments are completed.

TABLE V.

Concentrations of free amino acids in liver and urine of normal rats, estimated from chromatograms (amino acids are expressed as $\mu\text{g.}$ in 1 c.c. of urine and 1 g. of liver tissue.)

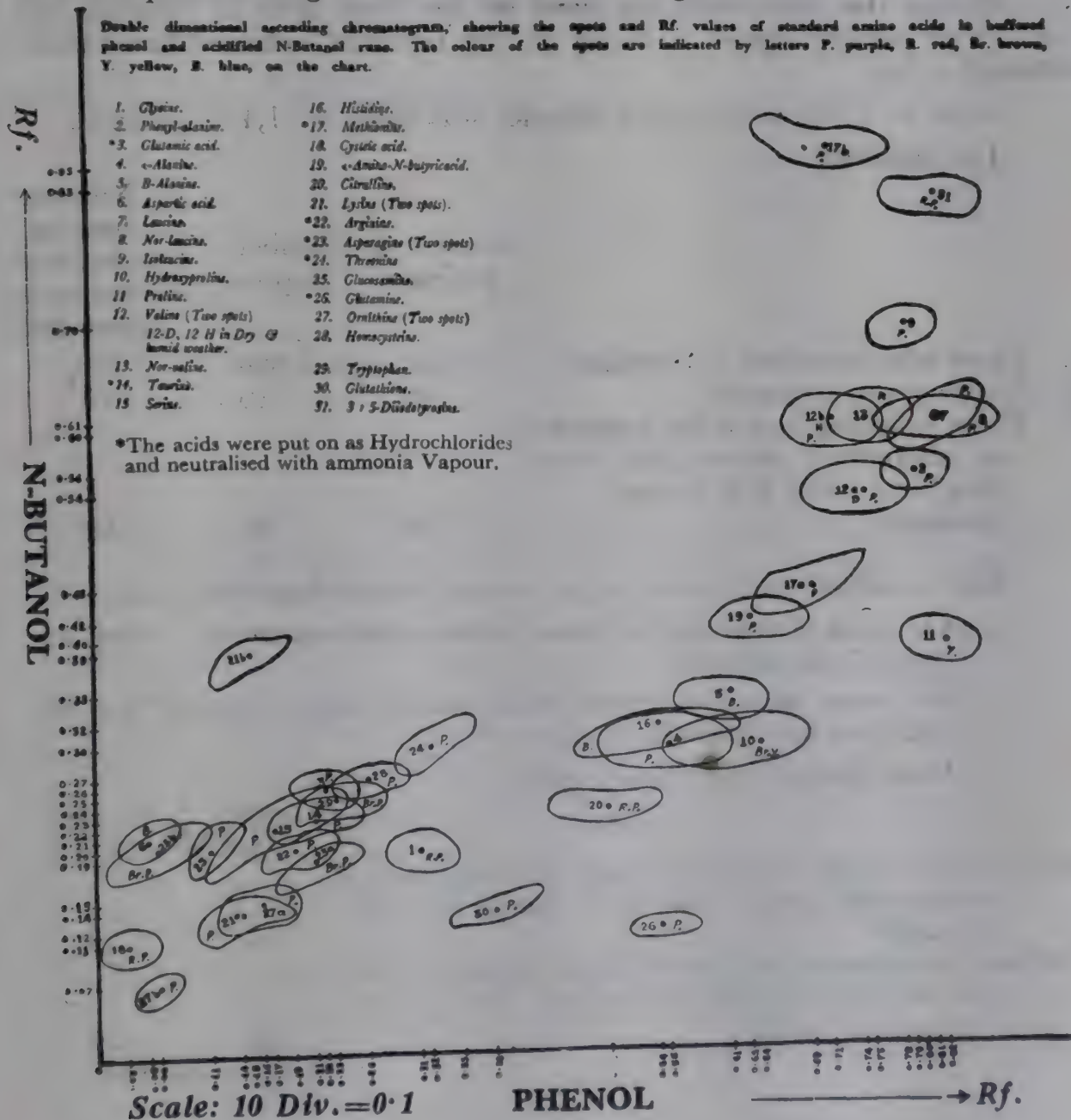
Amino acids	NUMBER OF EXPERIMENTS															
	Urine								Liver							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
1. Cysteic acids	400	391	397	405	410	390	401	394	1070	1099	1090	1080	1094	1086	1075	1084
2. Histidine	205	210	200	198	207	205	200	202	236·25	230	240	235	242	233	244	234
3. Tyrosine	50	50	52	56	49	52	48	47	157	160	155	150	153	150	155	148
4. Valine	50	47	46	50	53	54	51	52	118	120	123	123	121	124	116	115
5. Leucine	50	50	46	52	49	52	50	53	78·7	80	75	73	82	85	75	80
6. Aspartic acid																
7. Serine	150	155	148	153	155	148	154	154	472	470	480	465	470	486	471	484
8. Glycine																
9. Glutamic acid																
10. α-alanine	270	265	273	265	270	270	274	274	394	392	390	387	385	400	388	385

The spots due to aspartic acid, serine, glycine, glutamic acid and α -alanine were found to overlap each other on a circular chromatogram. The results in Table V are expressed as aspartic acid (for a spot due to aspartic acid, serine and glycine) and glutamic acid (for a spot due to glutamic acid and α -alanine). The above two amino acids (i) aspartic acid, and (ii) glutamic acid are well confirmed amino acids present on the chromatogram.

The values for cysteic acid, histidine and tyrosine reported here are appreciably higher than those reported in the literature. On the basis of small number of estimations done till date the value of our experiments cannot be vouchsafed. Further work is in progress to establish their values.

V. *Paper-partition chromatography of amino acids of liver homogenates and urines of rats treated with carbon tetrachloride (qualitative and quantitative studies).*

These studies are being conducted in rats which are injured with carbon tetrachloride. Together with histologic and histochemical enzymatic studies on the same livers, an attempt will be made to correlate the changes as a result of acute and chronic injury to the liver cell and study the variations from normal. As the experiments are still being conducted, it is not possible to give the results at this stage.



C. ALL-INDIA REGISTRY OF HEPATIC PATHOLOGY.

The Registry has had a successful year and is already showing signs of bright future. Till date, 160 cases have been registered. Of these 96 cases are from the Department of Pathology, S. N. Medical College, Agra, and 64 cases are from outside laboratories. These cases cover specimens of almost every type of liver disease and are complete with clinical histories, contributor's and Registry diagnosis, sections and in most cases blocks. It is a noteworthy achievement in a year's time and it is hoped that in another two or three years enough cases will be available covering all varieties of liver pathology to work out the morphological classification of liver diseases. The major contribution was from The Oswaldo Cruz Institute, Rio de Janiero, Brazil, which contributed 35 cases of liver diseases.

10. Studies of endometrial biopsies in cases of sterility with a view to estimate the incidence of latent tuberculosis of the endometrium under Dr. (Kumari) P. K. Malkani at the Lady Hardinge Medical Collge, New Delhi

During the year work was done on the same lines as reported last year. The present report is made on the material collected and results obtained so far.

Total of 1,918 endometrial biopsies was done on 1,491 patients.

This includes :—

		Number of patients	Number of biopsies	Number showing evidence of endometrial tuberculosis
1.	Those who consulted for sterility ...	1,411	1,838	106
2.	Fertile group (controls) ...	10	10	Nil
3.	Those suffering from acute pulmonary or abdominal tuberculosis atten- ding New Delhi T.B. Centre (controls) ...	70	70	Nil

Four hundred and twenty-seven repeat biopsies have been done :—

1. On cases of endometrial tuberculosis under treatment to note the response to therapy.
2. On cases in whom endometrial biopsy tissue obtained was insufficient for diagnosis, and on
3. Cases having anovulatory cycle.

	Number	Number positive
Ziehl-Neelson stain on histologically positive cases of endometrial tuberculosis for evidence of acid- fast bacilli ...	94	3
Cultures on endometrial tissue of histologically positive cases of endometrial tuberculosis (for growth of tubercule bacilli) ...	82	10
	On (57 patients)	

Menstrual pattern :

		Sterile group	Fertile group	Active extra-genital T.B. group	Endometrial tuberculosis group
1. Normal	414	10	40	13
2. Oligomenorrhœa	894	<i>Nil</i>	18	37
3. Menorrhagia	43	<i>Nil</i>	4	9
4. Metrorrhagia	2	<i>Nil</i>	<i>Nil</i>	1
5. Amenorrhœa	58	<i>Nil</i>	8	46
TOTALS	1,411	10	70	106

Amenorrhœa seems to be by far the commonest abnormality of menstrual pattern in cases suffering from endometrial tuberculosis. Four out of eight cases of amenorrhœa in the group of cases suffering from active pulmonary or abdominal tuberculosis had amenorrhœa due to lactation. Fifty-eight cases of amenorrhœa in sterile group includes 46 cases of endometrial tuberculosis.

Histological study of endometrial biopsy :

		Sterile group	Endometrial tuberculosis group
1. Proliferative phase	477	43
2. Secretory phase	880	9
3. Endometrial structure completely lost	54	54
TOTALS	1,411	106

Evidence of ovulation as detected by study of endometrial histology is seen in 62 per cent of the sterility cases and in 8·5 per cent in the group suffering from endometrial tuberculosis.

Tuberculous endometritis is reported to differ from tuberculous lesions in other parts of the body in the absence of caseation in the majority of cases. Our observations do not tally with the findings of other workers. In the series under study, 76 cases show caseation of varying degree and 45 out of these had amenorrhœa as menstrual abnormality. This can be explained by the fact that when menstruation occurs, endometrium is shed and when the endometrium regenerates during the next cycle, the new tubercles are about a month old ; hence caseation is likely to be absent. In cases of amenorrhœa, the tubercles are several months old, therefore caseation is frequently found. The periodic shedding of endometrium seems to cut short the formation of a mature tubercle. Out of 106 cases of endometrial tuberculosis, 46 had amenorrhœa and of these 45 show caseation. One case of amenorrhœa which did not show caseation will be further studied by repeat biopsies, as the endometrial tissue obtained at first biopsy was scanty.

BACTERIOLOGICAL INVESTIGATIONS.

Whenever histological evidence of tuberculosis is found in a specimen of endometrial biopsy, further evidence of tuberculosis is looked for by :—

1. *Ziehl-Neelson stain*.—Two more sections are prepared from the same paraffin block and stained with Ziehl-Neelson stain for evidence of acid-fast bacilli. So far 94 tissues have been stained but acid-fast bacilli have not been demonstrated in any section.

The following modified technique is being used now : Fita-Faraco method for staining acid-fast bacilli :—

- (i) De-paraffinize sections with two changes of a mixture of one part of olive oil and two parts of xylol.
- (ii) Drain, wipe back and sides, and blot section with filter-paper.
- (iii) Treat with (aqueous) lugols iodine solution and with aqueous five per cent sodium-thiosulphate solution to remove mercury precipitate if necessary. If not omit this step.
- (iv) Wash in water.
- (v) Stain for 15 to 20 minutes in Ziehl-Neelson's carbol fuchsin at room temperature.
- (vi) De-colorize for one to two minutes to a faint-pink colour with 1 c.c. concentrated hydrochloric acid in 99 c.c. 70 per cent alcohol.
- (vii) Wash in tap-water.
- (viii) Counter-stain with Mayer's alum hæmatoxylin for two to three minutes.
- (ix) Wash in tap-water till blue.
- (x) De-colorize with above acid alcohol, if necessary.
- (xi) Blot with filter-paper and keep in incubator at 37° C. overnight.
- (xii) Mount in clarite or similar synthetic medium.

Forty sections have been stained with the technique described above and so far acid-fast bacilli have been demonstrated in three sections.

2. *Culture of endometrial tissue*.—The patient is asked to return for repeat biopsy during the next cycle during the pre-menstrual week at which time the volume of the specimen is likely to be greatest. Patients suffering from amenorrhœa have repeat biopsies done at any time convenient to them.

The aim of this examination is to isolate tubercle bacilli by culture. The following procedure has been adopted for culture of endometrial tissue (by the Bacteriologist at New Delhi T. B. Centre).

MATERIALS AND METHODS.

Specimens of the endometrial biopsies were forwarded to the laboratory at the earliest opportunity in a five per cent solution of glycerol saline.

Cultures for the recovery of tubercle bacilli were put up on these specimens within 24 hours of receipt.

The specimens were subjected to a preliminary concentration technique so that a small amount of concentrated inoculum was obtained which was then transferred to the culture medium—two tubes containing culture medium being used for each specimen.

The American Treadue Society's 'potato-glycerine-egg medium', containing two per cent glycerine and malachite green in a dilution of 1 in 2,000, as inhibitory agent for the prevention of secondary organisms, was used. This is a solid medium.

Each specimen was thoroughly washed two to three times in sterile saline to remove all traces of glycerine and placed in a sterile pestle and mortar. Sterile sand was mixed with the specimen in equal quantity and grinding was carried out vigorously, sterile saline being added in small quantities at intervals.

After a two-minute grinding, additional saline was added to bring the volume to about 7 c.c. to 10 c.c. and the supernatant was transferred to a McCartney bottle. This was then treated with an equal amount of four per cent sodium hydroxide (sterile) and incubated for 30 minutes at 37°C.

After incubation, the specimen was centrifuged at 3,000 r.p.m. for 20 minutes. The supernatant was discarded, the sediment neutralized with eight per cent sterile hydrochloric acid in the presence of phenol red (0.04 per cent) as indicator. The neutralized sediment appeared straw yellow.

The sediment was then drawn into a sterile pipette and inoculated into 2 A.T.S. medium so that each tube received a minimum of 0.02 c.c. to 0.03 c.c. of the inoculum. The tubes were kept at slanting position for two to three hours to ensure a staunch settling of the bacilli on to the surface of the medium.

At the end of this period, the medium was transferred to the incubator (37.5°C. in the vertical position) and allowed to remain for a period of 12 weeks.

Observations for growth were made at the end of each week of incubation.

Specimen was reported negative if no growth was obtained at the end of eight weeks.

Eighty-two specimens of endometrial tissue of 57 patients have been cultured so far.

Ten specimens showed growth of tubercle bacilli in eight weeks. Reports of eight specimens are awaited.

GUINEA-PIG INOCULATION.

As guinea-pigs are not easily available, only seven endometrial biopsy specimens were inoculated in the guinea-pig.

The inoculation test was negative in all the seven.

MINIATURE RADIOGRAPHY.

1,932 patients at the sterility clinic have had miniature radiography of the chest done by the team of workers of New Delhi Tuberculosis Centre. This gave us an opportunity for detection of pulmonary

tuberculous lesions in the patients studied for evidence of endometrial tuberculosis. A large group of pregnant women has also had miniature radiography done.

A comparative statement of the two groups, so far as investigations up-to-date make it possible to state, is as follows :—

	Maternity group	Sterility group
Total examined	4,489	1,932
Number showing abnormal shadow ...	123 (2·74 per cent)	83 (4·30 per cent)
Number investigated (large films) ...	104	72
Destructively active	6	3
Clinically active	11	8
Inactive or healed	7	4
Probably tuberculous	8	6
Diagnosed as probably tuberculous on study of small films only	7	3
Total number of active tuberculous cases ...	32 (0·71 per cent)	20 (1·03 per cent)

The number of persons examined in the maternity group so far is 4,489 and in the sterility group 1,932. From amongst these 32 (0·71 per cent) in the maternity group and 20 (1·03 per cent) in the sterility group are likely to be cases of active pulmonary tuberculosis. The difference in morbidity incidence of pulmonary tuberculosis between the two groups is only 0·32 per cent and this is not significant statistically.

STATISTICAL CONSIDERATION BY STATISTICIAN OF NEW DELHI T.B. CENTRE.

Due to the small number of cases examined in the sterility group the incidence of 1·03 per cent has a large standard error of 0·23. The true incidence may lie anywhere between $1·03 \pm 2(·23)$ per cent, i.e. between 0·57 per cent and 1·49 per cent. The true incidence has to be determined with greater accuracy for final assessment. If a standard error of 0·125 is considered minimum for reasonable accuracy, statistically it would then be necessary to examine 6,600 women in the sterility group. If this figure is considered too high, it would be possible to obtain a standard error of 0·175 by taking sample of 3,500.

TREATMENT.

Seventy-four cases of endometrial tuberculosis have been receiving anti-tuberculous treatment. :—

Group I—Iso-nicotinic acid hydrazide	39
Group II—Streptomycin and PAS	27
Group III—Iso-nicotinic acid hydrazide and PAS ...	5
Group IV—Iso-nicotinic acid hydrazide, streptomycin and PAS	3
	<hr/> 74

Thirty patients of group I have been studied in detail. Repeat biopsies were done at intervals of one to two weeks during therapy. Follow-up records of biopsies repeated every month following caseation of therapy is also maintained.

The dosage used was 150 mg. iso-nicotinic acid hydrazide daily by mouth in three divided doses for a period of 8 to 12 weeks.

The following is the result of study of 30 cases treated with iso-nicotinic acid hydrazide. All the cases were free of endometrial tuberculosis at the end of therapy:—

		Number of patients free of endometrial tuberculosis (histologi- cal evidence)	
Week of treatment			
At the end of	1st	...	1
	2nd	...	5
	3rd	...	13
	4th	...	16
	5th	...	20
	6th	...	22
	7th	...	23
	8th	...	25
	9th	...	25
	10th	...	28
	11th	...	28
	12th	...	30

Follow-up of these 30 cases for five to ten months after cessation of therapy shows:—

- (i) Recurrence in two cases.
- (ii) Commencement of menstruation after a period of amenorrhoea of one and a half to four years duration has occurred in four cases. One of the patients whose endometrium was studded with tubercles and showed marked caseation before the commencement of therapy has now regular ovulatory menstrual cycles. This suggests that conception may be possible.

The results of group II treated with streptomycin and PAS are as follows:—

- | | | |
|---|-----|----|
| (i) Cases showing no response | ... | 10 |
| (ii) Cases showing healing | ... | 9 |
| (iii) Cases who have not reported for repeat biopsies | ... | 8 |

Study of groups III and IV is not complete.

INCIDENCE OF ACTIVE EXTRAGENITAL TUBERCULOUS LESIONS.

Three cases of endometrial tuberculosis show active pulmonary tuberculosis as proved by clinical and radiological examination.

Two cases of endometrial tuberculosis show evidence of abdominal tuberculosis on clinical examination.

INCIDENCE OF ENDOMETRIAL TUBERCULOSIS IN CASES OF STERILITY.

		Endometrial tuberculosis	Incidence, per cent
Total number of cases studied	... 1,411	1,106	7·5
Number primary sterility cases	... 1,101	66	6
Number of secondary sterility cases	310	40	13

The incidence of endometrial tuberculosis in secondary sterility is more than double the incidence in primary sterility. This suggests that cases of secondary sterility should be carefully investigated and any pelvic inflammatory lesions in such cases should not be dismissed as due to pyogenic infections, following abortion or delivery.

Seventy cases suffering from active pulmonary or abdominal tuberculosis had endometrial biopsy done in the pre-menstrual week. Not one of these showed evidence of endometrial tuberculosis.

11. Inquiry entitled fungus diseases of skin—identification of fungi under Dr. K. N. Saxena at the S. N. Medical College, Agra.

Up to this time 164 patients have been studied and their history noted on the clinical case sheet in all details. The following is the age distribution :—

Up to 10 years	9
11 to 20 years	51
21 to 30 years	49
31 to 40 years	20
41 to 50 years	21
50 to 60 years	12
Above sixty	2
			<hr/> 164 <hr/>

Out of these 164 patients, 140 are male and 24 female.

As regards occupation, the following is the observation :—

Farmer	27
Sweet seller	16
Fruit seller	7
Shoe maker	25

The rest have their occupation either as student, service, house-worker, etc.

Out of this total of 164, thirty-seven came from rural area and the rest from city.

There was history of some previous medication in 63 persons.

Clinically, a fungal patch arises as a papulo-erythematous lesion, usually oval or circular, on the smooth skin and it spreads peripherally.

The margins are thick and have scales. There is more erythema and inflammation as compared to *coccal* lesions.

Thirty-eight specimens of skin were cultured first in the Littman medium for primary isolation. The growth was then transferred to the Sabouraud's medium for confirmation. Sometime the skin pieces were taken for histological study also. Few cases of ringworm of nail were also studied.

The epidemiological study shows some interesting point as the adults are worst sufferers. Due to certain social customs, very few female patients came forward for treatment. As regards occupations, the ringworm infection is mostly seen in persons whose work is sedentary or in farmers who are closely associated with cattles. Though fungus infection seems to be quite prevalent in rural areas, very few care to have the hospital treatment as self-treatment or advertised treatment of fungus conditions of skin is a well-known fact.

In the laboratory diagnosis, the microscopical examination of the scrappings do not yield any definite diagnosis and hence this was abandoned. The scrappings, when collected for culture, did not show any successful result due to contamination. Hence, a wedge of skin of the affected area was incized and implanted on the Littmann medium for primary culture and isolation and this gives satisfactory result provided that all external skin contamination be avoided. Great difficulty was, however, encountered in inducing the patient and to cut a piece of skin from affected area as the ringworm of skin is regarded such a trivial ailment that people do not want to sacrifice a piece for the sake of diagnosis and treatment.

As regards the culture, it has been observed that Petri-dish and flat-flask culture proved a failure. Successful culture has been made on MacCartney bottle.

Up to this time, thirty-eight pieces of skin have been cultured. So far, ten cultures have been successful and the fungi that have been isolated are: *Trichophyton crateriform*, *Monilia albicans*, *Trich alba*, *Micro-sporum lanosum* and *Microsporum audouinii*. There are still nine more cultures under study and follow-up.

12. Enquiry on electro-retinography in vitamin-A deficiency under Dr. R. P. Dhanda, at the M. G. M. Medical College, Indore.

Since establishing the average of $\cdot 30\text{mV}$ as the current potential among normal Indian adults as reported in the first report last year, further investigations were carried on normal subjects under the age of 15 years. Twenty-two eyes of 19 subjects were investigated and it was surprisingly discovered that in 16 out of the 20 normal eyes the current potential varied from no response in those under 15 months age to *less than* $\cdot 20\text{mV}$ up to the age of 12 years. It would be interesting to establish, if possible, the cause of this low potential response by simultaneous vitamin-A level in the blood of children.

One hundred and twenty eyes of 84 patients suffering from clinical eye manifestations of vitamin-A deficiency of varying ages were investigated during the year under report, and it was discovered for the first time that

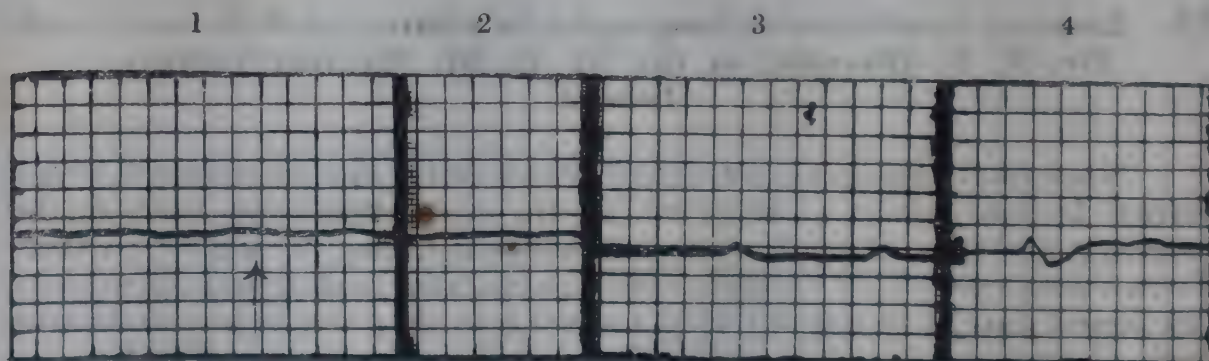
a stage comes when the vitamin-A level probably falls below a certain threshold when the symptom of night-blindness also sets in and the electro-retinogram suddenly gets extinguished. Absence of any response in cases of *Retinitis pignemntosa* was established, as of course also reported by Prof. Karpe of Sweden. Administration of massive doses of vitamin-A brought on clinical improvement very rapidly but the return of current potential was much slower than anticipated. Simultaneous estimation of vitamin-A level may reveal existence of interesting relationship between the clinical condition, the blood chemistry and the electro-retinographic response. It was also discovered that children are much more sensitive than adults not only in developing clinical manifestations of vitamin-A deficiency but also in losing the current potential much earlier.

The work done is reported in two parts :—

- (1) Recording of electro-retinograms in normals under the age of 15 years and to compare the results and determine the averages in different age groups.
- (2) Electro-retinography examination of patients suffering from various clinical eye manifestations of vitamin-A deficiency, the treatment of these conditions and recording of effects on the electro-retinographic response as well as on the clinical condition.

(1) *Investigations of normal children.*—Twenty-two eyes of 19 normal subjects under the age of 15 years were investigated during the period under report to find and compare the response with that of adults. Of these 4 were under the age of 15 months, in two of them the electro-retinogram had not developed at all, in the third the potential was $\cdot 05\text{mV}$ and in the fourth $\cdot 125\text{mV}$. From the age of three years, the response was invariably subnormal. Only in five eyes the potential recorded was about $\cdot 20\text{mV}$, while in one eye in a child of 13 years the response was only $\cdot 10\text{mV}$.

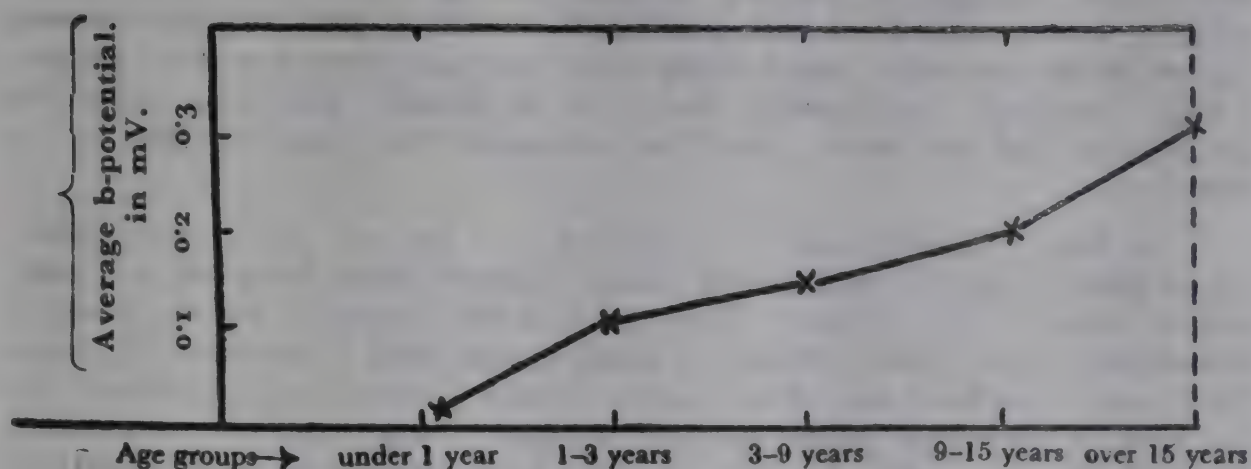
The following tracings are a few of those taken in different age groups :—



4 normals under the age of 15 years. Note increasing response with growing age
Calib. 1 cm. 1 mV.

- | | | | |
|---|--|-------------------------------|---|
| (1) R.-5 mths. b-pot. $\cdot 02\text{mV}$. | (2) R.-10 mths. ERG not developed yet. | (3) U. 4 yrs. 16mV . | (4) L.K. 13 yrs. b-pot. $\cdot 22\text{mV}$. |
|---|--|-------------------------------|---|

GRAPH



Showing b-potential in normals at different age groups.

The above tracings and graph and the following Table give an idea of how the current potential behaves and gradually develops more and more with the growth of the child and reaches the normal standard only after the age of 15 years.

Age groups	Number of eyes examined	Average b-potential
0 to 15 months ...	4 eyes of 4 cases	·04mV
15 months to 3 years ...	3 eyes of 3 cases	·12mV
3 years to 7 years ...	4 eyes of 5 cases	·12mV
7 years to 15 years ...	10 eyes of 7 cases	·20mV
Above 15 years ...	As per last years report	·30mV

The above findings, under fairly controlled conditions, create interesting field for further research into the aspect of sub normal response from the retina of younger age group normals. May be, it is due to higher threshold of vitamin-A level ; may be due to lower maintenance of vitamin-A level in the blood of children with utilizations by them of the larger amount of vitamin or it may possibly have something to do with the development and the functioning capacity of the retina in general and the rod cells in particular, of the children. Further investigations in the form of simultaneous estimation of vitamin-A in blood and recording the dark adaptation may throw some light to explain the above findings. Doing liver-function tests will also be a part of the investigations to exclude any liver pathology.

(2) *Investigations of persons with clinical manifestations of vitamin-A deficiency.*—In all 131 eyes of 92 patients were investigated. These investigations formed the following groups :—

- (i) Those suffering from xerosis alone (50 eyes of 32 persons).
- (ii) Those who complained of night-blindness alone and presented no objective pathological findings (seven eyes of six patients).
- (iii) Patients with xerosis *plus* night-blindness (71 eyes of 52 patients).
- (iv) Three eyes of two cases of keratomalacia.

Age group		Number of eyes examined with xerosis alone	Average b-potential
0 to 15 months	...	<i>Nil</i>	<i>Nil</i>
15 months to 3 years	...	3 eyes of 3 patients	·10mV
3 years to 7 years	...	8 eyes of 5 patients	·10mV
7 years to 15 years	...	2 eyes of 1 patient	·22mV
Above 15 years	...	35 eyes of 22 patients	·26mV

Treatment for these xerotic patches was given in different forms in six of the suitable cases. In one case sub conjunctival injection of oxycyanide of murcury was given, the patch disappeared and had not re-appeared for six months following the injection, even though this very patch was not affected by five dozen I.M. injections of vitamin-A, the patient had taken, before this local treatment. In another case subconjunctival injection of an aqueous solution of vitamin A under the patch gave similar results. In a third case 12 injections of vitamin A (I.M.) had to be given to make the patch disappear. The electro-retinogram, however, in all these cases remained unaltered as expected.

The above findings lead one to conclude that so long as the condition of xerosis alone persists and there are no other clinical manifestations of vitamin A deficiency, the electro-retinogram remains normal. One probable explanation of this is that vitamin A in blood must have a definite threshold up to which there are neither clinical manifestation of vitamin A deficiency apart from xerosis of course, nor is the electro-retinogram altered. Or it may be that probably xerosis as a clinical manifestation is the result not of direct vitamin A deficiency but an indirect manifestation. Further, investigations with simultaneous estimation of vitamin A in blood are likely to throw interesting light on this aspect and may afford a better explanation.

(ii) *Functional night-blindness*.—Only five patients complaining of an acquired nature without any clinical objective findings to account for it came under observation. In all the cases electro-retinogram was completely extinguished. Two of the cases were given massive doses of vitamin A for one week. Their symptoms improved but no action potential had yet developed. Further investigations with simultaneous estimation of vitamin A in blood would indicate the precise nature of these conditions.

(iii) *Xerosis with night-blindness*.—This group provided the largest amount of clinical material and here again history revealed that children were very susceptible to effects of vitamin A deficiency. Common history was that the child had xerotic patch for some time and had recently developed night-blindness. Quite often, night-blindness was precipitated by an infective fever or an intestinal disorder. Duration of night-blindness in most of these cases was short and the patient came for treatment for night-blindness and not for the xerotic patches. Again, onset of night-blindness was usually sudden. In case of adults the clinical material was mostly from the poor class.

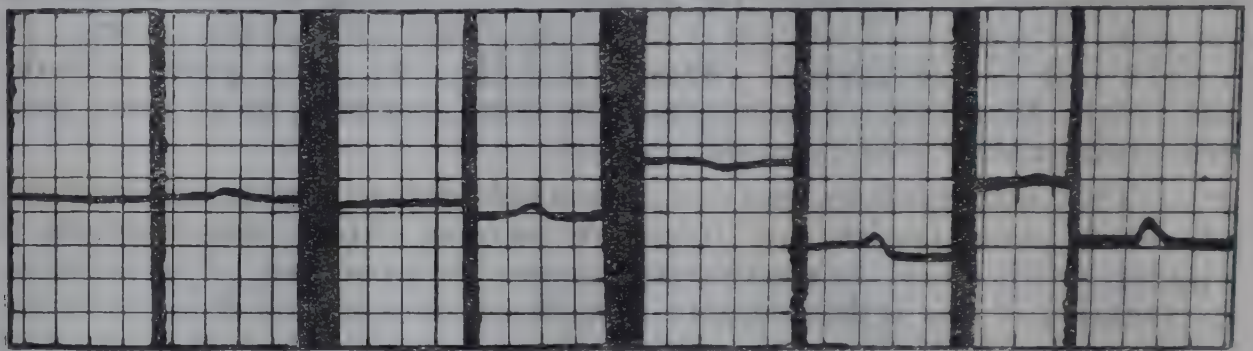
The 58 eyes of 40 patients formed the following age groups :—

Age groups		Number of eyes examined	Average b-potential
0 to 15 months	...	<i>Nil</i>	<i>Nil</i>
15 months to 3 years	...	6 eyes of 5 patients	All extinguished
3 years to 7 years	...	16 eyes of 14 patients	All extinguished except two
7 years to 15 years	...	11 eyes of 8 patients	All extinguished except five
Above 15 years	...	25 eyes of 13 patients	All extinguished except six

Twelve of the cases were followed up and treated with vitamin A injections. In most of the case night-blindness very rapidly disappeared and the action potential also showed a tendency to re-appear though rather to a lesser extent. Six injections of vitamin A (I.M.) 100,000 unit each, were necessary to initiate the onset of the return of action potential.

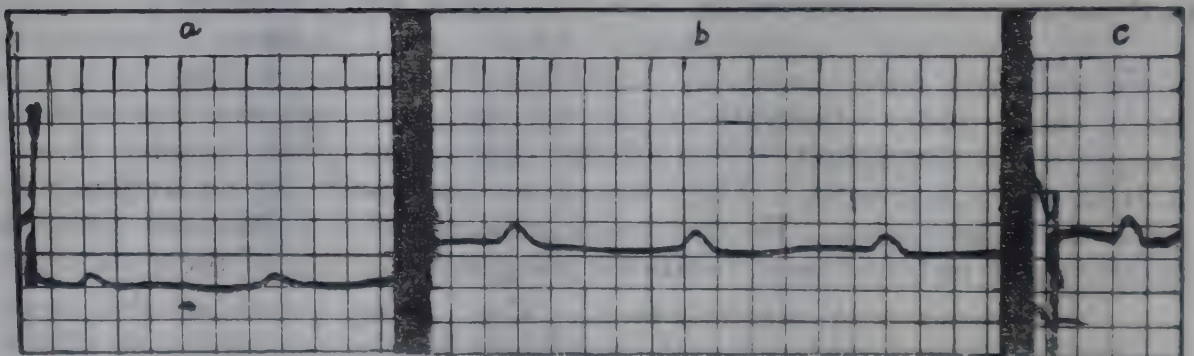
The following are a few of the tracings of these groups :—

No. 1 No. 2 No. 3 No. 4



4 cases of xerosis *plus* night-blindness before and after treatment with massive-A Inj. Note the extinguished ERG in each case before treatment & +b-potential after treatment.

1. O.P. 4 years 2 J. 3 years 3. J. L. 15 years 4. R.L. 25 years

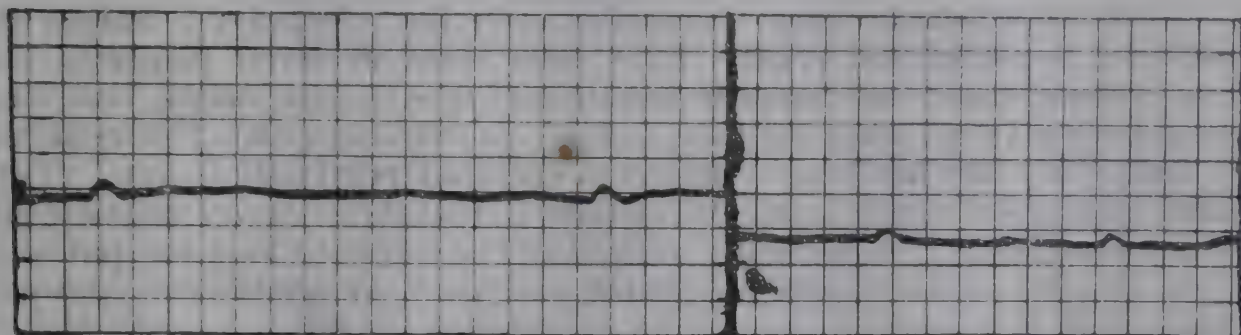


Miss N. 25 yrs. Xerosis 6 months with night-blindness. Calib. 1 cm.=1 mm, flash 1/4 sec.
(a) before treatment b-pot. - .15mV. (b) after 12 injs. of massive-A, b-pot. .25mV.
(c) after 18 injs. of massive-A when all symptoms disappeared. b-pot. 27mV.

Children up to the age of seven years proved very sensitive to vitamin A deficiency and out of 22 eyes only two recorded a current potential and that too less than .2mV with the rest all showing an extinguished electro-retinogram. Again, of the 36 eyes above the age of seven years

only ten recorded some b-potential and that too was only between $\cdot 05\text{mV}$ to $\cdot 20\text{mV}$. The above findings indicate the relationship between the onset of symptom of night-blindness as a manifestation of vitamin A deficiency and the sudden loss of the b-potential. To explain the few cases which showed some response, quantitative investigation as proposed may reveal the true picture. Existence of vitamin A threshold level in blood is, however, very strongly suggested by these findings.

(iv) *Keratomalacia*.—Only two case of keratomalacia, one of three years and the other of five years of age had b-potential $\cdot 17\text{mV}$ and $\cdot 20\text{mV}$, respectively, which is normal for this age group.



B.B. 5 yrs. A case of keratomalacia treated with massive-A inj. resulted in clinical cure. b-pot. $0\cdot 17\text{mV}$. i.e. same before and after treatment.

13. Inquiry on localization of a 'feeding centre' in hypothalamus under Dr. B. K. Anand at the Lady Hardinge Medical College, New Delhi.

Various workers have already shown that bilateral destruction of the ventromedial nucleus of the hypothalamus or its adjoining areas produces over-eating (hyperphagia) in different animals with consequent obesity. This obesity resembles that of the 'Frohlich's syndrome'.

While working at Yale University School of Medicine, U.S.A., the present worker was able to localize a small discrete area in the lateral hypothalamus of the albino rat on the same anteroposterior plane as the ventromedial nucleus, the bilateral destruction of which produces complete stoppage of spontaneous eating, and such an animal starves to death in spite of availability of food. This area was designated as 'feeding centre' as it controls the 'urge to eat'. It was also shown that possibly the ventromedial nucleus provides an inhibitory mechanism (satiety mechanism) for this. Results of these studies were published in the form of three papers.

The present inquiry was undertaken to attempt localization of such a 'feeding centre' in the hypothalamus of the higher mammals, cats and monkeys, and also to study in what manner, if any, the higher cerebral control in the higher mammals, modifies these responses. The work done so far is reported below.

Due to very high atmospheric temperatures in this part of the country, a very high mortality in the operated animals was experienced initially as the temperature regulation gets upset after hypothalamic lesions.

'This difficulty was got over after some time by making some cooling arrangements.

(1) *Hypothalamic lesions in cats.*—Electrolytic lesions in different regions of the hypothalamus have been produced so far in eight cats. In three in which the lesions are expected to be in the 'feeding centre', there was complete cessation of spontaneous eating, the animals being kept alive only by stomach-tube feeding. No change in food intake was produced from lesions in some other regions. More cats with lesions in different hypothalamic regions are still to be prepared, and the histological localization of the lesions has been undertaken now.

(2) *Hypothalamic lesions in monkeys.*—So far, electrolytic lesions in different hypothalamic regions have been done in seven monkeys. In two of these, there was complete cessation of spontaneous eating, and they would not eat even when the food was put into their mouth. This response is similar to what has been obtained in cats and previously in rats. Two other monkeys showed a modified response, in that there was no spontaneous eating even when food was freely accessible, but when the food was put into their mouths, eating would be elicited and the food would be swallowed. More work is required to venture an explanation for this. A much higher cerebral control in the monkeys as compared with the other animals studied, however, must be kept in view; when it is known that in man (highest cerebral control) eating can be elicited even when there is no appetite. In rest of the monkeys there was no change in the feeding responses. More lesions are still to be done, and histological localization has been undertaken.

(3) *Stimulation of hypothalamus in unanæsthetized animals by permanently implanted electrodes.*—This has so far been done in eight cats. Stimulation of lateral hypothalamic areas (feeding centre) increases the food intake, while there is no change in food intake when stimulating the other regions. More cats and at the same time monkeys will be stimulated. Histological localization of the points stimulated has also been undertaken now.

(4) *Blood-sugar changes as a result of hypothalamic stimulation.*—Results are inconclusive as yet, but it is apparent that over-eating by stimulation of 'feeding centre' is not due to hypoglycæmia (as suggested by some).

(5) *Hypothalamic control of ACTH secretion.*—In continuation of last year's work (reported separately) localizing the hypothalamic areas which control ACTH secretion by ablation studies in the rats. Changes in the eosinophile counts in the peripheral blood are being determined, as a result of stimulation of different hypothalamic regions, to localize the hypothalamic regions responsible for ACTH secretion. Results are inconclusive as yet, as histological localization not completed.

(6) Various autonomic and somatic responses as a result of hypothalamic stimulation are also being studied.

(7) Some of the animals, both after hypothalamic lesions, as well as after implantation of electrodes, never attain consciousness but remain stuporose. Others (majority) completely recover. It may be possible to offer some explanation after histological studies are completed.

14. Inquiry on the possibilities and scope of homologous venous and arterial grafts under Dr. P. K. Sen at the Seth G. S. Medical College, Bombay.

Forty-five grafting experiments have so far been performed. The work has been divided under the following headings :—

- A. Collection and preservation of grafts.
- B. Transplantation of the grafts into aortic defects (abdominal and thoracic).
- C. Retrograde aortography.
- D. Sacrificing of the animals and necropsy.
- E. Detailed examination of the graft including histological examination.

A. Collection and preservation of grafts.—Seventeen dogs were utilized for this purpose. Segments of varying lengths of thoracic aorta, abdominal aorta and inf. vena cava were taken out with meticulous aseptic precautions, their branches were ligated with 000 braided black silk and then preserved in a balanced electrolyte homologous serum solution at 4° C. Composition of preservative fluid is given in Table I :—

TABLE I.
Composition of preservative fluid.

Constituents	Mg./100 c.c.
NaCl	800
KCl	40
MgSO ₄	8
MgCl ₂	8
CaCl ₂	14
Na ₂ HPO ₄	6
KH ₂ PO ₄	6
NaHCO ₃	35·5
Glucose	100
Homologous serum	10 c.c.
Penicillin and streptomycin ...	5,000 units each

B. Transplantation of the grafts into aortic defects (abdominal and thoracic).—Aortic defects ranging from 5·8 cm. to 1·8 cm. were bridged in 40 dogs using 28 aortic and 12 vena-cava-grafts. This transplantation was done by two methods :—

- (a) Clamping and occluding the aortic flow during the whole period of grafting. By this method the aorta is occluded for 30 to 45 minutes.
- (b) By bridging the aortic defect by an in-dwelling polyethylene tubing carrying the graft over it. The tubing serves to maintain circulation through the aorta for the major part of the grafting operation. The tube is removed at the terminal part of the

anastomosis ; the rest of which completed after clamping the aorta. By this technique the aorta was occluded only for short periods of five to six minutes.

The details are as follows :—

1. Ten dogs have been utilized for homologous grafts in abdominal aorta below renal arteries as shown in Table II (p. 239).
2. Thirty dogs have been utilized for homologous grafts in thoracic aorta as shown in Table III (p. 240).
3. Five dogs have been utilized for bridging the aortic defect in thorax with polyethylene tube only as shown in Table IV (p. 241).

Grafting in the abdominal aorta carried a low mortality, but such grafts could only be done below the renal arteries. In case of the thoracic grafts immediate mortality was very high but after development of a method (b) of suturing the graft over an indwelling tube and thereby reducing total occlusion time of thoracic aorta the results have been encouraging.

At present attempts are being made to graft long segments (over 12 cm.) and to place such long grafts as parallel grafts to the aorta with a view to possible clinical application in treatment of aneurysms.

C. *Retrograde aortography*.—The grafted aorta has been visualized in three animals by retrograde aortography, injecting 70 per cent radio-opaque dye (dioduron cilag) through a polyethylene catheter inserted in the aorta via the femoral artery. The patency of the grafted segments has thus been proved.

D. *Sacrificing of the animals and necropsy*.—Two dogs have been sacrificed after 8 and 12 months from the date of grafting. In both the cases the graft was patent and functioning. Routine necropsies have been done on all post-operative deaths to study the condition of the graft and host-aorta and also to determine the possible cause of death.

E. *Detailed examination of the grafts including histological examination*.—Mensural and macroscopic studies are noted carefully at the time of grafting and at necropsy for comparison. Microscopic studies were done at two stages :—

(a) At the time of grafting in recipient dog.

(b) After the death or sacrificing of the recipient dog.

Histological sections from grafts and host aorta, have been prepared and are being studied by Dr. S. N. Kothare, one of the associates of this Inquiry.

The broad conclusions which can be drawn from studies carried on so far are itemized below :—

1. Homologous aortic grafts can be preserved by this method for a month or longer and successfully used to replace gaps in the host aorta.
2. Vena-caval grafts can be similarly used but the chances of success are less.

TABLE III.
Thirty grafts in thoracic aorta.

GRAFT						FATE OF ANIMAL		CAUSATIVE FACTORS						
Type	Number	Storage in days		Lenth in cm.		Fate	Surviving	Expired	Days from operation	Leakage		Shock and or clamp applied more than 30 minutes	Collapse of lung	Not known
		Max.	Min.	Max.	Min.					Next day	Delayed			
Aortic	21	35	5	5·8	1·8	Patent, functioning	1	20	270 1 8, 11, 15, 18 1 or 2 1 or 2 8, 10, 120	4	4	7	2	3
Vena-caval	9	17	2	3	1	Patent with aneurysmal dilatation		9	1 7, 53 1 or 2 1 or 2	2	2	3	2	

3. Changes occur in both arterial and venous grafts which consist in slow degeneration and fibrosis of the media but in successful grafts the endothelial lining remains smooth and intact.

4. Aneurysmal dilatation, thrombosis necrosis of the grafts and delayed leakage are the complications noted and these do not seem to depend on age of the graft or technique alone. Antigen-antibody reactions are involved, and further histological studies of the host aorta-graft junctions are needed to establish this.

5. Smooth-walled non-wettable tubes, such as polyethylene can be used to replace aortic defects for some days but there is real risk of ligatures cutting through producing hæmorrhage and/or occluding thrombosis.

6. The use of such a tube to maintain circulation through the aortic defect during grafting minimized risks of spinal damage due to anoxia and has a definite place in clinical application.

• TABLE IV.

Five polyethelene tube in thoracic aorta.

Dog number	Tube dimensions		Days of patency	Fate of animals	Post-mortem findings	
	Len- gth	Diameter				
		Ext.				Int.
67	2·5	0·8	0·7	22	Expired (23rd day)	Thrombosis
68	2·5	0·8	0·7	1	Expired (23rd day)	Hæmothorax slipping of tube
73	3·0	0·8	0·7	60	Expired (next day)	Thrombosis
78	4·0	0·9	0·7	6	Expired (185 days)	Hæmothorax cutting through of ligature
82	4·0	0·9	0·7	6	Expired (6th day)	Hæmotherax cutting through of ligature

15. Inquiry into endemic focus of schistosomiasis in India under Dr. R. K. Gadgil and Dr. S. N. Shah at the Grant Medical College, Bombay.

STUDIES OF THE OVA AND MIRACIDIUM OF *schistosomum hæmatobium*

For the study of ova they were obtained from patients suffering from this disease brought to Bombay from the endemic focus. The patients were passing through the acute stages of the disease.

The number of ova is very large in the last drop of urine. If urine is collected after the patient is fatigued then there is marked hæmaturia and the number of ova is also large. These eggs contain mature viable miracidia. The shells are oval at one end and tapering at the other end to a distinct spine. These ova measured from 110 microns to 170 microns in length, the average being about 135 microns in length.

The large number of ova has a length varying between 130 and 140 microns. The lowest measurement is 110 microns and the longest ova measured 172 microns. The measurement of the transverse diameter varies from 50 to 74 microns. The length of the spine varies between 8 and 11 microns. These ova are light-yellowish brown in colour and are semi-transparent. The movement of the miracidium can be easily seen through the shell.

If the urine containing the ova is diluted four to five times with water, the shell swells up and ultimately breaks open and miracidia come out. This occurs within 15 minutes of the dilution of the urine with water. In tap-water the hatching of miracidia occurs comparatively earlier than the well or rain water. If the urine is centrifuged and the deposit is kept at the frigidaire temperature for 30 to 40 seconds and then taken out, the miracidia immediately come out even without dilution of the deposit. If on the contrary the deposit is kept at incubator temperature the hatching process is not affected. The ova remain viable at room temperature for eight to ten hours only, while if these are kept at frigidaire temperature they remain viable for six days. If the urine is kept for some time undiluted the movements of the miracidia in the shell become sluggish and ultimately these die out. This observation has bearing on the infection experiment. For infection purposes the ova should be as fresh as possible so that the miracidia hatched out are quite vigorous to penetrate the snail.

The miracidium after hatching out of the egg shell spreads out and during this process it remains sluggish. This spreading out or enlargement occurs due to release of pressure to which it is subjected in the egg shell. Then it becomes very active and swims away vigorously in water almost in a straight line till deflected by an obstruction in its course. It tries to attack and penetrates such a particle and when it fails it moves away from it.

In the hatching out process after the eggs have been put in water for some time the miracidium in the egg shell becomes active. The movements of the miracidium are of a wriggling type in the shell. During this process the cilia move vigorously and fine granules are seen floating about in the egg shell. First the movements are not continuous but occur at intervals and then the movements become more or less continuous till the egg shell breaks open and the miracidium comes out. The egg shell breaks open always in the longitudinal diameter and not the transverse diameter of the egg shell.

The miracidium changes its shape during movement. When killed with formalin it is pear shaped. The posterior end tapers down to a blind end, while the anterior ends in the mouth cavity. The body of the miracidium is covered by ciliated epithelium. There is a non-ciliated band completely encircling the organism in the palae of excretory pores. The oral cavity is continuous with the primitive gut. On both sides of this primitive gut lie the anterior salivary glands. The other pair of salivary glands lie distal to the posterior ends of the anterior salivary glands and open on the antero-lateral margins. There are two pairs of flame cells. These flame cells are observed best when the movements of the organisms are restricted. These flame cells flicker and resemble the flame of a candle. The flickering is continuous till the organisms die out. These flame cells are connected with tubules opening through a single

pore on the postero-lateral margin. The germ cells arise from the germinal epithelium at the posterior end of the larva and are proliferated in the brood cavity. If the miracidia are kept in a test-tube half filled they are distributed throughout the various levels of the water. This finding again has bearing on the infection experiment. It is possible that even if the snails are completely submerged or at the border of the water-level it is possible for the miracidia to penetrate the soft parts of the snail.

LABORATORY STUDY OF THE SNAIL HOST IN THE ENDEMIC FOCUS.

In the initial phases of investigation as regards the probable intermediate host—the snail was identified as *P. obesi* (Philipi) by Zoological Survey of India. Some specimens of the snails were sent to Dr. Mandahl Barth, W.H.O. expert, for opinion. Dr. Mandahl Barth expressed surprise that a prosobranch snail was found to be a vector for this fluke infection. The report on the identification of the snail is that—this snail is not a typical *P. obesi* but should be grouped under the same class. Nothing is much known about the biology of the snail. It was suggested that the snail survey of the locality during different seasons should be carried out as this snail does not belong to the group of African snails (*Bullinus* and *Physopsis*) which serve as intermediate host of the infection. This opinion was corroborated by Professor Buckley of the London School of Tropical Medicine.

The work was carried out as follows :—

The survey of the locality for snail fauna was carried out during the months of October 1952, December 1952, January 1953, March 1953, and May 1953. During this survey it was noticed that the *P. obesi* occur in large numbers in live streams of the village throughout these months. Some dead shells of snails were collected from the surrounding fields. These shells belong to the *Indoplanorbis exustus* group of snails, but no live snails of this type were found in the infected stream. This rivulet was searched up and down the stream for a distance of two and a half miles where it joins the other two rivulets from the neighbouring villages. The snail fauna of the other two rivulets is the same. While studying the snail fauna observations as regards the habitat and biology of the snails were made. It was noticed that the snails occur near the bank of the stream and remain submerged in the shallow water. The colour of the shell of the snail is that of the stones in the rivulet. The younger snails were found to be attached to decaying leaves. Large number of snails were found semi-stagnant water along the bank of the rivulet.

Numerous attempts to maintain these snails in the Laboratory were made keeping the above-mentioned observations in mind. The experiments carried out are as follows :—

1. Ground aquaria $3' \times 1\frac{1}{2}' \times 9''$ deep were built and 500 snails were placed in these aquaria. Tap-water was used. The mortality of these snails was very high at the end of ten days. Well-water was used in one aquaria, still the mortality was high. Water was dechlorinated, still the mortality was high.
2. Two glass aquaria $24'' \times 12'' \times 16''$ were prepared. These aquaria were used with sand at the bottom in which were planted some water plants. Aquaria were filled with tap-water and allowed to

- stand for eight days and then 50 snails were placed in each. Even in these aquaria mortality of the snails remained very high.
3. Small glass aquaria were made and snails were fed with lettuce twice a week with change of water after every 24 hours. The snails lived in these aquaria for three weeks and then died.
 4. When these experiments failed an attempt was made to keep the snails alive in running water. Fifty snails were put in a shallow enamel tray and a continuous running water stream was maintained for six hours a day. These snails were quite happy at the end of 35 days. This experiment clearly shows that a running stream is essential for the maintenance of the snails.
 5. A cement aquaria was built 30" × 12" × 9" with irregular sloping surface inside and a syphon arrangement was made. In this aquaria water plants were put and a running water stream four hours a day was maintained. One hundred snails were put in this aquaria for breeding purposes.
 6. Another two tanks were prepared again and some larvicidal fishes were put along with snails for experiments. In these tanks also a running stream of water was maintained for three to four hours a day.
 7. Glass aquarium was prepared again and some larvicidal fishes were put along with the snails. In this aquarium snails belonging to *Indoplanorbis exustus* group and *Limniolæ leutole* group were put along with few *P. obesi* for observation and breeding.
 8. Three cement aquaria have been built and snails are maintained at Gimbvi village with arrangements of a syphon system of running water. The water for these aquaria is used from the stream after filtration.
 9. Infected young snails are being maintained in small glass aquaria using rain water here as well as in Gimbvi.
 10. One tank aquaria is kept on the ground in the open air. In this aquaria also rain water is used for aeration.

THE INFECTION EXPERIMENTS WITH *Paludomus obesi*.

In the first group of our experiments we have used the adult snails. These snails were tested intermittently for cercarial emission for a period of one month. When they were found to be negative they were taken up for infection experiments. They were exposed to miracidia in the following manner :—

1. Large number of snails and large number of miracidia kept together in an enamel tray overnight. Next day they were transferred to their proper aquaria.
2. Five to twenty miracidia were kept with a snail individually in a small Petri-dish overnight. The snails thus infected were transferred to their proper aquaria next day.
3. Large number of snails were exposed to large number of miracidia. Such exposures were repeated on alternate days for three to four exposures.

4. Some snails were infected in the very aquarium in which they were living since a month by pouring the urine in aquarium tank. The water in this aquarium was kept stagnant for a period of 24 hours and then the running water was started.
5. Same as No. 4 above but three to four exposures were given on alternate days.

All these experiments were unsuccessful. Almost 80 per cent of snails died in four weeks' time and the rest when tested for cercarial emission failed to show any furcocercaria.

These experiments were repeated with snails of about half the size of adult snails. The result was again the same and not a single snail showed the presence of furcocercaria.

By this time we received the much-needed guidance in our work from Dr. Wright. On his advice we directed our work on young snails.

At this stage of our work we had not been able to keep the snails alive in our aquaria for more than a period of two months. Hence there was no breeding and we did not have any laboratory-bred snails for our experiments.

We collected the very young snails from the river. As to their exact age we had no idea, hence we grouped them according to size. Only those snails which were less than 2.0 mm. in length were selected for experimental purposes.

We dissected about three hundred such young snails. None of them showed the presence of any cercarial fauna. Then a group of snails were tested intermittently for cercarial emissions for a period of two to four weeks. Only those snails which were found to be completely negative were exposed to miracidia for infection.

These were exposed to infection in the following manner.

1. Miracidia were placed in an aquarium at the rate of about ten miracidia per snail. The water was kept stagnant for 24 hours and then they were aerated.

2. The same as No. 1 above but such three to four exposures were given on alternate days.

3. Some snails were infected individually in a test-tube with five to twenty miracidia per snail.

4. About 50 snails placed in a small Petri-dish and exposed to miracidia at the rate of ten miracidia per snail, kept overnight, and the snails were returned to their aquaria the next day.

The mortality among these snails was very high, almost 80 per cent of snails dying in the first week and the remaining in the second week. Only five snails survived a period of four weeks and that only when we took the advantage of monsoon and built up an out-door aquarium for these snails. One snail of this group became positive. The furcocercaria recovered from this snail were brevifurcate and of a different morphology to the one we had recovered before from adult snails.

It is probable that the high mortality among the snails is due to either heavy infection or unsuitable environment. In our further experiments we are reducing the number of miracidia to one to three per snail and trying to improve the environment as far as possible.

CLINICAL SURVEY OF THE DISEASE.

There was definite doubt in our mind that this disease must be affecting the whole village and not limited to one locality only. Cercarial antigen was procured through W.H.O. from Dr. Blair, Southern Rhodesia, to carry out skin test. We carried out this antigen skin test in the local primary school group of eight children picked out from the different localities of the village. Six groups were tested. The skin test was positive in about 70 per cent of the children tested. This test was confirmed by examination of the urine deposit. In about 85 per cent of positive skin test cases ova were seen in the urine, while in the remaining cases urine examination was negative. This proved that though the prevalence of the disease is maximum in the locality near the stream, it is wide spread in the whole village of Gimbvi.

These children are under-nourished and look anæmic. The degree of hæmaturia differs in each individual case. Many of the children had dermatitis. Stool examinations were negative for schistosome ova in all these cases. There was no splenomegaly or enlargement of liver in any case. We have already pointed out that the hæmaturia, the predominant symptom of the disease, disappears at the age of puberty. In the adults who gave history of the disease, the main complaint was low aching pain in the lumbar region and discomfort during micturition. The adult worm lives in the tissues for ten to twelve years. On these basis it can be explained that these children get the infection at the age of five and when the worms die at the age of 15 to 17 years the hæmaturia disappears. It is equally important to note that there are no cases of re-infection seen after the age of puberty. It is quite possible that they get immunized against this disease. Four cases of school-going children were brought to Bombay by us. These cases were useful for us to carry out the infection experiments of the snails and morphological study of the miracidia and ova. Cystoscopic examination was done on two cases. The cystoscopic examination showed extensive ulceration of the mucose of the bladder and deposits of eggs were seen at places in the bladder wall. An old case of the disease was investigated. A descending pyelography was done. The radiograms revealed bilateral hydronephrosis dilatation of the ureters and lineal calcification of the bladder wall.

Three of these cases of school-going children were treated with injections of fontarin (antimony preparation). It was seen that after the fourth injection the hæmaturia becomes less and with further injections the ova becomes non-viable. Then the hæmaturia disappears completely but cystitis persists. It slowly disappears at the end of two months.

16. Studies of Indian poisonous snakes under Dr. P. J. Deoras at the Haffkine Institute, Bombay.

This scheme was taken up with a view to see if the snakes of the four most common species in India could be kept in the snake farm under conditions akin more or less to those obtainable in nature, with the object of increasing their longevity and also with the object of studying their breeding habits. It was also intended to use the snake farm in the Institute to rear the young ones of Russell's viper, Cchis, etc., to adult stage by experimenting with different kinds of food, as it has been found

that snakes when kept in captivity in wire cages do not live longer than three to four months on an average. If as a result of keeping snakes in the snake farm their longevity materially prolonged, it was expected that each snake would yield more venom and this would solve to some extent the problem of availability of snake venoms for manufacture of antivenine. If attempts to breed the snakes and rear the young ones to adult stage in the snake farm succeeded it would solve the supply problem of venom for good.

The snake farm in the Institute was put into operation on 2nd January 1953. It consists of four different sections, each section being reserved separately for cobra, Russell's viper, krait and echis. The different species were kept separately in the different compartments as it has been found that two different kinds of species do not live in the same compartment without harming each other.

STRUCTURE.

Snake farm.—In the Institute compound a snake farm was built in 1936. This farm consisted of four 40' \times 40' pits surrounded by 5' high wall and an intervening moat. There was no shade inside the farm and the snakes took shelter in cement concrete domes kept in the farm on earth. The domes got hot in summer and the earth became muddy in rains. The snakes probably did not get adequate shelter and the use of the farm had to be discontinued after some time.

Last year a trellis-work over iron girders at a height of 15 feet was constructed to cover this snake farm with the idea of providing shade by growing creepers over the trellis-work. Adequate vegetation of perennial green shrubs was also planted and the farm put in operation from 2-1-53. Experiments were immediately started to find out how best each snake could be kept in each section of the farm so that the animal is not affected by sun and rain and at the same time it would be safe for workers to move about the place.

Cobra farm.—It was noticed that cobras would not take shelter in the domes. Experiments were, therefore, done to keep these animals in mud tunnels, stone crevices, mud pots kept open and mud pots kept buried and covered. It was seen that these snakes preferred the mud pots that were buried in cement platform 3' \times 3' \times 4' and partially covered by wooden planks. The entire structure was protected against sun and rain. The cobras are now living inside these pots situated in the cement platforms. In bright summer these shades had to be covered by thatch to keep away the sun. When working, a wire-gauze over the pot mouth made the farm safe for workers. It was found that these adult snakes preferred mice to frogs as their food. This had to be given once a week at night time in order to keep away birds. Cobras take to the food first in comparison with other snakes. A number of frogs (*Rana tigrina*) are also kept in the moat surrounding the farm.

Krait farm.—These snakes would not live in pots as did the cobras. In the experiments it was seen that they preferred thin crevices between stones kept near very moist places. Wooden boxes with inlets kept near the water channel, and small brick rooms with stones inside were preferred. These snakes are, therefore, now seen to live in small 2' high rooms and stone collections over the cement platforms. They prefer extreme humidity,

narrow spaces and dark surrounding. They also like small mice given in the night once a week.

Russell's viper farm.—In the experiments for keeping these snakes it was noticed that Russell's viper preferred earthen mounds with slabs of rock inside. Xerophytic plants were put in this rockery and during the rains it was covered by a matting. The snakes lived throughout the summer in this structure. The heavy rains of Bombay did not, however, give adequate protection to the mound on all sides so that by the end of July the snakes got buried in the mud and had to be pulled out. They take mice as their food.

Echis farm.—This was started on the 3rd of August, 1953 as the snakes were received in July. In this farm, two cement concrete rooms full of stones and red soil and covered by tin covers harbour a major number of these snakes. Water plants are put in large numbers in the moat and thorny bushes in the farm. These snakes are seen to rest on these plants. Tiny molluscs, millipeds and very young frogs have been liberated in the farm for their feeding and the snakes seem to like them.

Behaviour in the farm.—Cobra seems to be very sensitive in rushing back to its hide-out. Krait and Russell's viper do not mind the noise or disturbance. Snakes seen staying out of hide-out during broad day light were found to be sick and they died in due course. It was noticed that younger snakes are not allowed by the older ones to stay with them in the same hide-out. In fact the young ones ran away. Russell's vipers were found to eat their young ones. Snake of a different race is not tolerated in one group of hide-out. This was clearly seen in Russell's viper farm, where the Madras vipers firstly would not stand to Bombay climate and secondly the fierce Bombay vipers actually drove them away, with the result the Madras vipers were lost one by one. There was a keen competition between the different adult snakes in the farm for survival and a number of younger Russell's vipers died in this fight. This was not seen in the case of kraits. In the case of cobras, two males were kept with a female in a pot and one of the males was recovered dead in two days.

Rearing and breeding experiments.—Previously, if any young ones were born to Russell's vipers in captivity, they were found not to live for more than a month. In the case of echis also the same was the case. By changing their food they were found to live longer in captivity. The young ones of a Russell's viper lived for six months in the Laboratory, feeding on young frogs. It was also possible to keep ten echis alive for six months in cages after feeding them on tiny frogs. This was the first attempt to keep them alive for so long in cages. In the snake farm the young ones of Russell's viper lived for three months but were later attacked by adult snakes.

Natural breeding habits of these snakes being comparatively unknown, experiments were done to see if it would be possible to induce ovulation by the administration of pituitary hormone. Experiments were done first on frogs (*Rana tigrina* and *Bufo vulgaris*). Anterior pituitary gland from frogs and sheep were grafted on frogs but they were without effect. Antutrin, 0.25 c.c. at a time, was given per 1,000 gm. weight of frogs on alternate days for four turns. Seven days after the injection, vocal sacs and pads were developed in male frogs. In toads spermatozoa were given out after the first injection. These injected frogs changed colour to yellow much before the monsoon and also developed vocal sacs. Egg spawns

were laid in the farm five months after first injection. Similar injections were given to cobra, krait and Russell's viper. In the last case spermatozoa were recovered from the cloaca after the fourth day of injection. There was no appreciable change seen in the case of cobras. Five months after the injection, two kraits gave out ten eggs each. These eggs did not hatch but shrivelled away. In the case of Russell's vipers, seven young were born in two batches in the farm. These young ones lived hunting the young frogs which were timed to hatch a few days earlier. These snakes—young ones, also preferred the earthen mound where the other older Russell's vipers lay hidden. Two of the adult Russell's vipers that had died revealed in one case fangs, and in other case nearly digested body of a young one, confirming the belief that the adults ate the young ones. The section is not yet set. Observations are being made in nature and food habits studied so as to make these available in the farm.

Adequate amount of perennial vegetation is just planted to give sufficient shade and privacy for hiding without at the same time exposing the workers to any risk. Table I (p. 250) gives the percentage mortality in the snake farm as compared to the same in snakes kept in cages. The average comparative output of venom per snake for the last six months is given in Table II (p. 251). The snakes were milked only once in a month in the farm and twice a month in the cages.

Table I shows that the percentage of mortality so far as cobra and krait snakes are concerned is lower in the snake farm than in cages. Though it is only one month, but in case of echis the mortality in the room was on an average 12 per day, while in the farm it is only 1.66 per day. Table II shows that the venom output of all the three snakes is higher in the farm than in the room for the same period this year and also last year.

Biochemical studies.—This work was started in the middle of June 1953. The problem to be worked out was to see whether there is any inhibitor in the blood of snakes that makes them tolerate appreciable amounts of other venom as well. Studies were also undertaken to determine the composition of the following constituents of the blood of snakes as also that of frog, mice and rabbits: Hæmoglobin, total blood volume, erythrocytes count, leucocyte count, calcium, magnesium, inorganic phosphorous and phosphatase activity.

Studies on the neutralizing action of snake blood and serum have so far shown that 0.25 c.c. of Russell's viper serum neutralized 0.1 mg. of Russell's viper venom injected intravenously. Russell's viper snakes on an average give about 14 c.c. of serum and on this basis they may be able to neutralize 5.6 mg. of other Russell's viper venom injected intravenously. Further work to confirm this is being done on Russell's viper and other snakes as well. Experiments are being done to isolate different globulin fractions from the serum to find out if possible, which of them are responsible for this neutralizing activity. The snake serum was fractionated with ammonium sulphate. The different fractions, euglobulin, pseudoglobulin and albumin, are under test for venom neutralizing activity. To find out if there are any inhibitors in the serum, attempts have so far proved negative to trace any tryptic activity qualitatively. This has also to be confirmed.

Morphological studies.—The elongation of the body, the loss of external appendages and the rectineal movement in snakes has brought about in their wake modification of the internal organs. The compression of these internal organs has apparently altered the typical bilateral symmetry and these modifications are an ample evidence of the ecology and behaviour of each of these snakes. The detailed morphological studies of the internal organs have shown that it is possible to explain the behaviour of the animal on this basis and also give a clue to confirm the classification which is often based on varying external characters.

Cobra and krait snakes both show one complete right lung and very tiny rudimentary left lung attached to the trachea. There is no tracheal lung in cobra and krait. The blood vessels are modified on this basis. The adrenals are small over thin kidneys and the penis is paired. Both these snakes belong to family *Cobubridæ*. In the case of Russell's viper and echis there is only one lung with a tracheal lung attachment and a complete absence of even the rudimentary left lung. The pulmonary arteris and veins are accordingly modified. The adrenals are long, thick and alveolar, situated over globulated kidneys. The paired penis bifurcates at the tip in both. These snakes belong to *Viperiade*. The long sustained hissing of Russell's viper and the cool calculated daring with which it strikes may be explained by the presence of the long lung extending all over the body with a tracheal attachment and probably the large adrenals may have to do something with the cool daring of the animal. Figures of these internal organs are being drawn to fill in the lacunæ on the morphological knowledge of these snakes.

TABLE I.

Average percentage mortality in cobra and krait snakes kept in cages and in the snake farm.

Snakes	AVERAGE PERCENTAGE MORTALITY		
	In cages in snake room during the period of 7 months from 2-1-52 to 1-8-52 and 2-1-53 to 1-8-53		In snake farm during a period of 7 months from 2-1-53 to 1-8-53
Cobra ...	41·66	42·8	39·9
Krait ...	69·23	37·6	18·0

TABLE II.

Average venom output in grammes per snake kept in cages in the room and snake farm during a period of 7 months of 1952 and 1953.

Months	1953 SNAKES KEPT IN CAGES IN THE ROOM AND IN THE OPEN SNAKE FARM						1952 SNAKES KEPT IN CAGES IN THE ROOM			Remarks
	Cobra		Russell's viper		Krait		Cobra	Russell's viper	Krait	
	Room	Farm	Room	Farm	Room	Farm				
January	0·149	0·289	0·097	...	0·022	...	0·130	0·085	0·010	Snakes are milked once in every 15 days in the room and every 30 days in the farm.
February	0·141	0·120	0·087	0·187	0·008	...	0·098	0·076	0·005	
March	0·169	0·199	0·010	0·390	0·005	0·013	0·109	0·085	0·005	
April	0·146	0·208	0·103	0·134	0·006	0·010	0·119	0·101	0·007	
May	0·155	0·192	0·145	0·172	0·010	0·023	0·160	0·105	0·007	
June	0·155	0·355	0·126	0·144	0·006	0·131	0·156	0·086	0·007	
July	0·200	0·382	0·131	...	0·001	...	0·160	0·103	0·008	
Average yield per snake	0·143	0·245	0·111	0·188	0·010	0·022	0·133	0·091	0·007	

17. Inquiry on experimental study of the mechanism of action of *Vibrio cholerae* on intestinal mucous membranes under Dr. S. N. De at the Nilratan Sarkar Medical College, Calcutta.

The object of the Inquiry is to see if the massive outpouring of fluid brought about by *V. cholerae* or its products is associated with increased permeability of intestinal capillaries to proteins and to try the effect of anti-cholinergic, sympatholytic, de-toxicating and other drugs having no direct action against the vibrio on this fluid loss when the more exact mechanism underlying the increase of capillary permeability may be defined.

Last year it has been reported that from experiments on ligated loops of small intestine in rabbits, *V. cholerae* appears to alter the permeability of intestinal capillaries to proteins and that paralysis of parasympathetic nerve-endings by atropine cannot check this increase of permeability.

During the period under review, phenergan—the potent antihistaminic drug, priscol—a sympatholytic drug, British Antilewisite, citrate and oxalate (to remove calcium ions) have been tried and these have been found to be incapable of restoring the alteration of permeability induced by *V. cholerae*.

The following drugs were tried during the year :—

(i) *Phenergan* (*promethazine hydrochloride*).—Twenty mg. to 40 mg. (as two per cent solution) of the drug per kilogram of body-weight was injected subcutaneously every seven to eight hours so that each rabbit received four such injections. The animal was sacrificed one hour after the last dose and twenty-four hours after the introduction of *V. cholerae* into the ligated loop of small intestine. Ten rabbits were experimented upon and the quantity or quality of accumulated fluid or the gross or microscopic appearance of the intestinal wall was not different from what was found in control animals which received no phenergan.

This potent anti-histaminic drug seems to have no effect on the action of *V. cholerae* or its products which suggests that liberation of histamine is not possibly related to the increase of permeability induced by *V. cholerae*.

(ii) *Priscol* (2 *benzyl*—4 : 5-*imidazoline hydrochloride*).—Ten rabbits received subcutaneously 0.8 c.c. to 1 c.c. (8 mg. to 10 mg.) of one per cent solution of the drug every seven to eight hours as with phenergan. No effect on evidences of altered intestinal permeability by *V. cholerae* was seen. Blocking the adrenergic sympathetic fibres has no effect and *V. cholerae* appears not to act through these fibres.

(iii) *British Antilewisite* (2 : 3-*di-mercaptopropanol*).—This drug is known to combat the increase of capillary permeability induced by phosgene and by heavy metals and has been shown to inhibit the SH enzyme which is involved.

Ten rabbits received 0.5 c.c. to 1.0 c.c. (25 mg. to 50 mg.) of five per cent solution of the drug per kilogram of body-weight subcutaneously every seven to eight hours after *V. cholerae* was introduced into the ligated loop of intestine. No difference from the control animals receiving no injection of BAL was noticed in the loop after twenty-four hours. The toxin of the vibrio does not seem to act on the capillaries through SH enzymes.

(iv) *Removal of calcium ions.*—Calcium ions have been found to be essential for the hæmolytic activity of *V. cholerae*. The effect of removal or inactivation of calcium ions with citrate and oxalate was, therefore, investigated to see if calcium is necessary also for its toxic activity on the intestinal capillaries. Five experimental rabbits received 10 c.c. of 10 to 20 per cent sterile solution of sodium citrate subcutaneously every seven to eight hours as in the previous procedures. In another five rabbits 1 c.c. of peptone-water medium from which calcium has been removed by addition of potassium oxalate in 3.5 per cent concentration and filtration was used as the vehicle for *V. cholerae* into the lumen of the loop. These measures did not succeed in preventing the accumulation of fluid inside the ligated loop of small intestine.

18. Studies of the behaviour of cavities in the lungs with a view to find out their causes under Dr. T. J. Joseph at the Lady Linlithgow Sanatorium, Kasauli.

An analytical study of the clinical records, serial X-ray photographs and fluoroscopic diagrams of cavities which showed unusual behaviour during their clinical course or under collapse therapy in 14 cases already discharged from the Kasauli Sanatorium has been made.

Nine cavitory cases of a similar nature now in the Sanatorium are being studied in the same way.

Thoracoscopy and cauterization of pleural adhesions were done in three cavitory cases and records made in regard to the changes in size, position and fluid contents of the cavities, and also in regard to the onset of atelectasis in the lung tissue surrounding the cavities.

Bronchoscopies were done in four cases with a view to assess the condition of bronchi in relation to the behaviour of cavities. While the larger bronchi could be studied by this means, in no instance could the entry of a bronchus into a cavity be demonstrated with the bronchoscope available.

Attempts made in two cases to make lipiodol introduced through the trachea enter cavities by positioning the patient suitably, failed in both cases. Three c.c. of lipiodol was introduced into two cavities in two patients. One of these cavities appeared to be a tension cavity. The patients were tilted so as to get the cavity emptied through the draining bronchi. In the non-tension cavity the lipiodol was seen under the fluoroscope to drain into the trachea. This did not happen in the tension cavity. The lipiodol was, however, coughed but completely from both cavities in 24 hours.

One cavity was entered with a trocar and canula, and the interior of the cavity examined with a thoracoscope. No definite information could be obtained regarding the condition of the draining bronchus. Later, the same cavity was surgically laid open through the chest-wall and a naked-eye inspection of its interior made. No wide-open draining bronchi could be seen. Two raised puckered spots in the lining membrane of the cavity could be seen through the centre of one of which bubbles of air escaped into the cavity when the patient held his breath and strained. Five c.c. of lipiodol introduced into the cavity could not be made to enter the draining bronchi by tilting the patient suitably.

The impression obtained by these clinical studies is that while such studies continued over a longer period may help, quick and conclusive evidence to explain the behaviour of pulmonary cavities can only be obtained by the production and study of experimental cavities in animals.

Adams and Worwald of the Departments of Surgery and Pathology of the University of Chicago in their work on 'The treatment of pulmonary tuberculosis by bronchial occlusion' (*Jour. Thoracic Surg.*, **3**, 6, p. 633 August 1934) used dogs, monkeys and goats for the production of experimental cavities. They introduced 0.1 c.c. to 0.2 c.c. of a heavy suspension of human tubercle bacilli sub-pleurally (through a thoracotomy) or intra-bronchially (through a thin rubber catheter passed through a bronchoscope) for the production of cavities ; and for occluding bronchi, they used the method of cauterizing the bronchial mucosa with a 35 per cent solution of silver nitrate through a bronchoscope. It is proposed that these techniques which seem to be the best hitherto known for these purposes be used in the present research also. With the advent of potent anti-tuberculosis drugs, it may be possible to control disease in animals better than it was possible before.

With a view to experimental studies on these lines, an X-ray-Penetrable airtight wooden box has been made with glass windows for naked-eye observations and an exhaust pipe through which the pressure in the box can be varied. With the help of this box, the radiography and bronchography of animal lungs in an inflated state and under collapse, is being standardized. The mechanical changes, such as kinking, which may occur in bronchi when pulmonary lobes are displaced or collapsed, are also being studied with the help of this box.

19. Inquiry into the clinical and pathological changes that are present months or years after apparent recovery from 'Kwashiorkor' or 'nutritional dystrophy' under Dr. S. T. Achar at the Medical College, Madras.

A series of 70 children out of the cases of nutritional dystrophy otherwise known as Kwashiorkor admitted to the children's departments of General Hospital and of Women and Children's Hospital, Madras, in the past few years was taken up for follow-up studies during these few months (59 cases of the year 1950 series and 20 cases of 1949 series). Out of these, only seven were available for re-admission, follow-up, clinical appraisal and liver-biopsy studies. Of the rest, i.e. 72 cases, 21 cases were reported dead some time after discharge from hospital. The rest of the 51 cases could not be traced either due to incorrect address given in the old case sheets or the tenants having left the place, new addresses not known. Only one sibling of the reported dead cases was available for clinical appraisal and investigation during the period.

The findings of the follow-up cases are given in Table I.

Side by side with the above work, some new cases admitted for nutritional œdema in recent months have been studied with regard to liver histology and attempts are being made to do a follow up study of these after discharge from the hospital.

Also, three cases of what looked like hepatic fibrosis of nutritional origin are being studied clinically and by serial liver biopsies. The findings of these three cases are given in Table II.

SUMMARY.

It is too early to draw any conclusions from the data available so far. All that could be said, however, at the present stage is :—

- (i) That quite a significant proportion of cases of Kwashiorkor have died after discharge from the hospital.
- (ii) That height, weight and general physical growth of some of the survivors appear to be retarded in most of the cases reviewed so far.
- (iii) That liver changes are absent in some, while in a few there have been very interesting changes in the liver histology.

<p>er biopsy studies (sections of a biopsy specimen stained by & E., toluidin blue, P.A.S., tori (s reticulum, van son and Sudan IV in ylene glocol).</p>	<p>REMARKS</p>
<p>arently normal liver histo- except minimal cellular ration in portal areas.</p>	<p>This girl of 5 years has the weight of a 1-year old child and height of a 3-year old.</p>
<p>normal liver histology.</p>	<p>The boy of 4 years has the weight of a 2½-year old normal boy and height of a 3½-year old.</p>
<p>at distortion of lobular archi- re. Most of the parenchyma show change suggestive of protein deficiency. Cells have rarefied cytoplasm reduction of A. particles—(tol. blue). Very few show loss of fat, little or no fatty vacuola- tion (Sudan IV does not show). Cellular infiltration+ in around vascular tracts+ portal thickening+stellar arrangement of periportal fibrous tissue+.</p>	<p>This boy of 5½ years has the normal weight and height for his age, but his liver is palpable and histology shows protein- deficient cells and periportal scarring.</p>
<p>arently normal liver histo-</p>	<p>This boy of 3½ years has the weight of a 2½-year old normal child and height of a 3½-year old.</p>
<p>lobular pattern seems to be maintained. Parenchymal cells and there, in groups shows rarefied cytoplasm or vacuolated cytoplasm. Most of the maintained their nuclei, no vacuolation. Reticular thickening. Cellular infiltration in portal areas.</p>	<p>A boy of 6 years has the weight of a 3-year old child and height of 3½-year old. This boy is stunted in growth and his liver though not palpable shows changes suggestive of protein deficiency.</p>
<p>normal liver histology.</p>	<p>This girl of 5 years has the weight of a 2½-year old normal and height of a normal girl of 5 years.</p>
<p>biopsy refused.</p>	<p>This girl of 4 years has the weight of a 2½-year old normal child and height of a 4½-year old child.</p>

1. The first part of the paper

is devoted to a discussion

of the various methods

which have been used

in the study of the

20. Studies in the development of pancreatic diabetes under Shri P. B. Sen at the University College of Science & Technology, Calcutta.

Sulphhydryl compounds were found to reverse the diabetogenic action of alloxan. The diabetogenic effect of an intraperitoneal injection of alloxan was suppressed when this was preceded by an intravenous injection of glucose, fructose or mannose. The action of glucose was found to be highest in this respect. The potency of mannose was slightly less, and fructose was least potent among these sugars. Galactose did not, however, possess any such protective action. Glucose, mannose and fructose in presence of ATP can act as substrates of non-specific mammalian hexokinase. There is a separate kinase for galactose. The protective action so far investigated is related to the affinity of these sugars for hexokinase. To examine this postulate various sugars and sugar derivatives were tested for their power of preventing alloxan diabetes.

Rats weighing 100 mg. to 150 mg. were used for these experiments. They were injected intravenously with sugars or sugar derivatives five minutes prior to an intravenous injection of alloxan (40 mg./kg.). Animals were considered to be diabetic, if within the experimental period of seven days, sugar could be detected in the urine, and the blood sugar was higher than 180 mg. per cent for two consecutive days (*see* Table I).

TABLE I.

Effect of pre-administration of sugar and sugar derivatives on the development of diabetes when intravenous injection of 40 mg./kg. of alloxan was given.

Number of rats used	Sugars and sugar derivatives	Doses	Number of diabetic rats	Remarks
6	Cane sugar	5 g./kg.	6	No protection.
6	Lactose	5 g./kg.	6	" "
8	Maltose	3 g./kg.	6	25 per cent protection
8	Maltose	5 g./kg.	0	100 per cent "
6	d-arabinose	5 g./kg.	6	No protection.
8	l-arabinose	5 g./kg.	8	" "
6	d-xylose	5 g./kg.	6	" "
6	l-sorbitol	5 g./kg.	6	" "
6	d-mannitol	5 g./kg.	6	" "
6	d-glucose amine hydrochloride	2 g./kg.	6	" "
6	Sodium gluconate	2 g./kg.	6	" "

The effect of these sugars were studied to determine whether the structural peculiarities of these sugars bear any relationship with their diabetes-preventing power. None of these sugars or sugar derivatives afforded protection except mannose, which in 3 g./kg. dose gave 25 per cent and in 5 g./kg. dose gave full protection to the rats injected with

alloxan. Due to the presence of the enzyme maltose in the tissues, maltose is the only di-saccharide that is hydrolysed within body and may be partially utilized when injected intravenously. The 'protection' obtained with maltose may be due to increase of glucose concentration in blood. This has not yet been confirmed.

Another angle from which this investigation was carried was to study how far the substances affording protection from alloxan diabetes would also affect the concentration of sulphhydryl compounds in blood. Glutathione content of blood is diminished in different types of diabetes. There is a diminution of glutathione concentration immediately after a diabetogenic dose of alloxan. The conditions that reduce the glutathione content of blood also increase the susceptibility of animals to alloxan diabetes. On the other hand, protection from diabetes has been observed to be afforded when animals were injected with glutathione and other SH compounds. The effect of alloxan injection on the glutathione concentration of blood has been shown in Table II :—

TABLE II.

The variation of reduced glutathione concentration of blood in rabbits following intravenous injection of five per cent alloxan at 17 mg./kg.

Weight of male rabbit	REDUCED GLUTATHIONE CONCENTRATION IN MG.				
	After 12 hour fast	2 minutes after alloxan	10 minutes after alloxan	30 minutes after alloxan	3 hours after alloxan
1,300	30	7	3	18	45

When the effect of intravenous injection of glucose was examined in animals in which diabetes has been produced with alloxan, a favourable response was obtained in certain cases. The study of a large group of animals and the effect of repeated dose of glucose injection will establish the real significance (Table III).

TABLE III.

The effect of intravenous injections of 25 per cent glucose at 1 g./kg. dose on the reduced glutathione concentration of blood in rabbits, rendered diabetic with alloxan (five per cent) solution at 170 mg./kg.

Weight of rabbits in grammes	MG. PER CENT OF REDUCED GLUTATHIONE (A) AND GLUCOSE (B) IN BLOOD									
	Fasting 5 minutes after glucose				15 minutes after glucose		45 minutes after glucose		24 hours after glucose	
	A	B	A	B	A	B	A	B	A	B
1,180	18	542	14	960	20	922	30	868
1,220	18	750	16	954	56	925	40	900
1,160	28	308	18	596	26	549	28	550	30	413
1,150	24	350	16	600	30	520	26	490	20	400

Intravenous injection of 25 per cent glucose at 1 g./kg. 20 to 25 minutes prior to intravenous injection of alloxan (five per cent) at 170 mg./kg. indicates a definite protection and improvement in the glutathione concentration of the blood (Table IV). A limited number of experiments that were carried with fructose and mannose indicated similar protection and improvement in glutathione level in blood (Tables V and VI).

TABLE IV.

The effect of intravenous injection of 25 per cent glucose at 1 g./kg. dose, 20 to 25 minutes prior to alloxan injection on the reduced glutathione concentration of blood of rabbits.

Weight of rabbits in grammes	PERCENTAGE IN MG. OF REDUCED GLUTATHIONE (A) AND GLUCOSE (B) IN BLOOD OF RABBITS									
	Fasting 12 hours		20-25 minutes after glucose		10 minutes after alloxan		30 minutes after alloxan		24 hours after alloxan	
	A	B	A	B	A	B	A	B	A	B
1,200	26	125	32	540	24	480	52	386
1,190	38	146	70	560	58	480	60	495	34	150
1,300	38	100	76	491	...	471	60	471	33	100
1,260	36	140	72	500	60	498	50	471	28	128

TABLE V.

The effect of intravenous injection of 25 per cent fructose at 1 g./kg. dose 20 to 25 minutes prior to alloxan injection on the reduced glutathione concentration of rabbit's blood.

Weight of rabbits in g.	Percentage in mg. of reduced glutathione					
	Fasting 12 hours	20 minutes after fructose	3 minutes after alloxan	10 minutes after alloxan	30 minutes after alloxan	3 hours after alloxan
1,357	31	24	24	29	61	56
1,800	57	80	40	45	convul- sions	...
1,400	45	65	55	80

TABLE VI.

The effect of intravenous injection of 50 per cent mannose 2.5 g./kg. dose, 20 to 25 minutes prior to alloxan injection on the reduced glutathione concentration of rabbit's blood.

Weight of rabbits in g.	Percentage in mg. of reduced glutathione					
	Fasting 12 hours	20 minutes after mannose	3 minutes after alloxan	8 minutes after alloxan	45 minutes after alloxan	3 hours after alloxan
1,190	33	35	24	22	53	The animal had convulsion and died

The effect of alloxan on the enzyme of the tricarboxylic cycle was investigated. Alloxan was found to inhibit the oxidation of pyruvate by liver homogenate preparation very slightly. Glucose was unable to prevent the action of alloxan (Table VII).

TABLE VII.

O₂ uptake in cmm. Substrate—0.01 mol pyruvate, final concentration.

Expt.	15 minutes	30 minutes	45 minutes	60 minutes
Control ...	35	52.5	75	88
Alloxan ...	25	42.5	53	60
Alloxan plus glucose	17.5	33	51	57.5

Formation of citrate.—The first step of the tricarboxylic-acid cycle is the condensation reaction of pyruvate and oxalo-acetate to give rise to citrate. This condensation reaction can be studied as an isolated step by treating the enzyme with barium ions which stop the tricarboxylic-acid cycle at citric acid stage. When barium-treated enzyme was treated with alloxan no further inhibition was obtained. This indicated that alloxan could not inhibit the formation of citrate though Co A. A SH enzyme is involved in this step (Table VIII).

TABLE VIII.

O₂ uptake in c.mm. Substrate—pyruvate malate.

Expt.	15 minutes	30 minutes	45 minutes	60 minutes
Control ...	32.5	63	83	95
Barium chloride ...	12.5	23	32.5	38
Barium chloride plus alloxan	10	20	29	40

Formation of alpha-ketoglutarate from citrate.—This step was studied with the help of arsinite which inhibits the oxidation of pyruvate beyond alpha-ketoglutarate stage. Alloxan was found to have no action on this stage (Table IX).

TABLE IX.

O₂ uptake in c.mm. Substrate—citrate.

Expt.	15 minutes	30 minutes	45 minutes	60 minutes
Control	28	72·5	100	115
Arsinite	8·5	26	35	42·5
Arsinite plus alloxan ...	8	24	32·5	40

Oxidation of alpha-ketoglutarate.—0·033 M malonate completely inhibits the oxidation of alpha-ketoglutarate beyond succinate stage. Alloxan was found to be incapable to inhibit malonate-treated liver homogenate enzyme preparation (Table X) :—

TABLE X.

O₂ uptake in cmm. Substrate—alpha-ketoglutarate.

Expt.	15 minutes	30 minutes	45 minutes	60 minutes
Control	42·5	66	77·5	93
Malonate	12·5	15	22	25
Malonate and alloxan ...	10	17·5	22·5	27

Oxidation of succinate.—The oxidation of succinate to fumarate can be isolated for study by shaking the enzyme preparation at 40° C. for about 20 minutes before tipping in the succinate. Alloxan was found to cause about 50 per cent inhibition of this pre-heated enzyme preparation (Table XI).

TABLE XI.

O₂ uptake in c.mm. Substrate—succinate.

Expt.	15 minutes	30 minutes	45 minutes	60 minutes
Control	40	67·5	93	120
20 mins. pre-heating 40° C.	17	30	35	45
Heating plus alloxan ...	8	13	17·5	25
Heating plus alloxan plus BAL	10	17	23	27·5

The above observation was confirmed by another technique. 0·033 M arsinite has been utilized by Krebs to block the oxidation of succinate by pigeon-breast muscle beyond the fumaric-malic-acid stage. This can also

be used on liver homogenate for the same purpose. It was found that alloxan would inhibit the oxygen uptake by 50 per cent in arsinite-treated systems when succinate was used as substrate (Table XII) :—

TABLE XII.
O₂ uptake in cmm. Substrate—succinate.

Expt.	15 minutes	30 minutes	45 minutes	60 minutes
Control	63	107·3	138	170
Arsinite	10	21	25	30
Arsinite and alloxan	5	10	11	12·5

Oxidation of malate to oxalo-acetate.—0·05 M cyanide combines with the oxalo-acetic acid formed from malate thus prevent the condensation of pyruvate and oxalo-acetate to form citrate. The action of alloxan on this single step reaction indicates a slight stimulation. This may be due to the disappearance of cyanide. The exact cause has not yet been investigated (Table XIII) :—

TABLE XIII.
O₂ uptake in cmm. Substrate—malate.

Expt.	15 minutes	30 minutes	45 minutes	60 minutes
Control	35	64	75	86
KCN	8	25	35	43·5
KCN and alloxan	25	42·5	53	57·5

From the above observations we can show that alloxan inhibits the oxidation of pyruvate by interfering with the conversion of succinate to pyruvate in the tricarboxylic-acid cycle.

21. Investigation on the value of aqueous humour examination in the ætiology of iritis and iridocyclitis under Dr. Lalit P. Agarwal at the S. N. Medical College, Agra.

CLINICAL MATERIAL

A queous puncture was performed in irdocyclitis cases. In all 29 punctures were done. The cases were selected from the Ophthalmic Wards of the S. N. Hospital and the Leper Asylum, Agra. Later, it was decided to conduct an experimental study first. Four punctures in normal rabbits have been done to find out the normal cytology of aqueous humour of rabbit's eye.

There were tremendous difficulties in the beginning in the selection of cases as the patients more often than not refused to undergo the procedure but later on mild persuasion succeeded. The work on the inquiry could

not be started early on account of numerous difficulties met with in the setting up of an experimental laboratory and a Papanicolaou Staining Laboratory and in procuring Amsler's and Verrey's apparatus for aqueous examination.

The punctures were made on the line of Amsler and Verrey. The aqueous was drawn in a tuberculin syringe by a needle specially designed for the purpose by Amsler. The fluid thus obtained was utilized for cytopathological examinations as given below :—

1. *Total-protein estimation*.—One drop of the fluid was allowed to fall from the syringe into five per cent phenol solution in a watch-glass on a dark background. Any protein present coagulated and the amount present was assessed by the colour of the coagulum (plate blue 0·3 to 0·5 per cent, transparent blue 0·5 to 1·2 per cent, opaque white more than 1·2 per cent).

2. One drop of the fluid was placed on a clean slide and allowed to spread by hanging (Drop Smear Technique). The smear thus obtained was immediately fixed in a solution of equal parts of alcohol and ether. The smear was stained by Papanicolaou method.

3. One drop was centrifuged in a small-bore capillary tube of Verrey at a speed of 1,500 revolutions per minute for ten minutes. The centrifugate was stained by Papanicolaou technique. After some cases the method was given up as it had no advantage over the drop-smear technique developed in this series. Besides this, the centrifugation procedure is cumbersome.

4. One drop was used for total cell count just like w.b.c. count but without the addition of any other fluid.

5. Another drop was used for identification of any bacteria by Gram's staining.

6. One drop was used for culture on blood-agar medium.

7. The last drop was used for observation under dark-ground illumination.

OBSERVATIONS.

The cases so far examined have been of the following types :—

1. Post-operative iritis	5
2. Acute iritis	4
3. Iritis with hypopyon	4
4. Chronic iridocyclitis	5
5. Doubtful iritis	2
6. Traumatic iritis	2
7. Leprous iritis	5
8. Syphilitic iritis	2
				—
				29
				—

PROTEIN ESTIMATION.

The rough method of protein estimation has given only an idea of the stage of iritis without contribution to its ætiological factor. Since the stage of iritis can be clinically diagnosed, this crude method of estimation has not been of much value. It is felt that a more elaborate biochemical method with higher accuracy may of be some value.

TOTAL CELL COUNT.

In normal rabbits the total cell count varies between 0 and 5 cells. In all types of iritis an increase in cell count has been found during the active stage. Acute leprous iritis, iritis with hypopyon and post-operative Iritis have been shown the maximum cell count. In one case the cell count was as high as 175/c.mm. of fluid.

Differential cell count.—It is much too early yet to draw any definite conclusions from a small series of 29 punctures though it is felt that this procedure may give an indication of the ætiology of iridocyclitis. Cylindrical ciliary epithelium has invariably been found in cases of endophthalmitis phako-anaphylactica together with an increase in total-protein contents. Sometimes corneal endothelial cells can also be demonstrated. Presence of ciliary epithelium in recognizable form has not so far been reported. In leprous iritis cases keratinized cells probably of iris epithelium can be constantly demonstrated along with varying numbers of lymphocytes and monocytes. Besides this, there is a considerable amount of fibrin deposit in these cases. A large number of polymorphonuclear cells are found in cases of iritis with hypopyon. They exhibit a tendency towards spontaneous clot formation. In traumatic iridocyclitis after perforation of globe there was a preponderance of polymorphonuclear leucocytes but there was no tendency towards clot formation, thus differentiating it from iritis with hypopyon. In acute stages of the disease the count has been always higher than in the chronic and sub-acute stage. No findings of any significance were obtained in syphilitic iritis but in view of only two cases observed in this series one need not despair. No phagocytic cells were found in this series nor could cells from the reticulo-endothelial system be demonstrated.

The cells that were observed in this study are :—

1. Polymorphonuclear leucocytes.
2. Lymphocytes.
3. Mononuclear cells.
4. Pigmented cells.
5. Corneal endothelial cells.
6. Ciliary epithelial cells.
7. Keratinized iris endothelial cells.
8. Lysed and degenerate cells.

Bacteriological examination.—In view of the small series in this study the absence of any bacteria in the findings is insignificant.

Darkground illumination.—Complex yet beautiful pictures have been seen under darkground illumination. In several cases of acute iritis, specially of the leprous variety, luminous spots in intense agitation were observed.

It was at first thought that they were bacteria, but smear and culture examinations belied the contention. They were perhaps protein particles in Brownian movements. Cholesterol particles and lipoid material should also be seen but it is felt that the darkground examination should be correlated with biochemical assay to be of any diagnostic value.

EXPERIMENTAL WORK.

Four experimental punctures on rabbits have yielded the following data :—

1. Total cell count ... 0 to 5 cells/c.mm.
2. Differential cell count ... Monocytes, lymphocytes.
3. Darkground illumination ... Optically empty.
4. Protein content ... Less than .3 per cent.
5. Bacteriological findings ... Negative both in smears and cultures.

It is now proposed to cause iritis and iridocyclitis experimentally in rabbits with known micro-organisms and to study their aqueous humour citopathologically for a period of three months at weekly intervals. The work is already in progress. This type of study is likely to help in arriving at some definite conclusions in experimental animals. The information thus obtained will be utilized for evaluation of results in human beings.

22. Studies on electro-encephalogram (brain-wave record) of normal and abnormal human beings with special reference to Indian conditions under Dr. N. N. Das, at the University College of Science & Technology, Calcutta.

SYNOPSIS.

Normal human subjects of different age groups from 9 to 80 years have been selected for electro-encephalographic recording. Fifty cases were recorded upto now. Of these mostly are students, teachers and medical men. There are a few records which show a certain pattern of brain waves which may be comparable to normal records of other Western countries with slight faster frequencies in a group of subjects. There are a few cases which gave a normal history, but the wave patterns show some abnormality of slow waves. The results indicate that there seems to be some variations in the EEG normogram in Indians. This difference might be due to different climatic conditions or due to the difference in nutritional status of the subjects. These should, however, be confirmed after taking a large number of records.

A group of human subjects has been selected who have no history of convulsive seizure, birth injury, head injury or encephalitis, etc. They had no serious illness or general weakness of the body. The subjects were of different age groups ranging from 9 to 80 years. They include both male and female sexes. The normal EEG (electro-encephalogram) of each person is unique. The concept of normal is useful only when it is related to a functional competence. Although each person is electro-encephalographically distinct from his neighbour in some particular, the

primary concern is not with variations as such but the types of electro-encephalographic differences that are related to inadequacy.

In classifying adult electro-encephalograms the dominant frequencies and the percentage of time that each is present are all taken into account in subjective evaluation of the electro-encephalogram. Wave form, synchronism and topographical distribution are also considered. EEG with dominant $8\frac{1}{2}$ to 12 per second activity and no abnormal slow or abnormal fast activity are classified as normal for adults. Notched waves with U or M shapes are not considered abnormal, for although they are unusual they do not correlate with dysfunction.

The subject lies quietly on the bed with eyes closed when recording is done. Before recording a number of eight electrodes are placed on different surfaces of the scalp. Two frontals (RF, LF), two premotors (RPM, LPM) and two occipitals (RO, LO) and two temporals (RT, LT) have been fixed. Two ear-lobe grounds are connected with electrodes for having a body connection which is not electrically active as brain. This is required for monopolar recording. Biopolar recording is done by scalp-to-scalp connection. After marking areas on the scalp with a skin pencil, electrodes are placed on the scalp. The Grass model type electrodes consist of a silver concave disc of 9 mm. diameter connected to a thin plastic covered wire, the other end having a pin to plug into the electrode board. For fixing up the electrode, a small amount of jelly is applied to the concave surface and the marked point on the scalp is cleaned with ether and alcohol to make it free of oil. The electrode is held in position with the help of a special holder and a few drops of collodion is applied all round. A compressed air jet is allowed to play on the liquid collodion which dries it in a very short time and the electrode is fixed and if the subject moves his head, it would not affect him in any way. After placing the scalp electrodes, ear-lobe electrodes are fixed with scotch tape and frontal electrodes are fixed with adhesive tape. This procedure takes about $\frac{1}{2}$ an hour. When this is completed the subject is allowed to lie on a bed in the sound-proof screened room. Near the head on the wall the electrode board with number is placed and this is connected to the machine by a cable.

When the patient is lying quietly with eyes closed the records are taken with the EEG machine. First monopolar recording is taken, and then several combinations of biopolar recording are taken. The speed of the paper is adjusted to 30 mm. per second. This speed is approved by the International Board of Electro-encephalographers. At this speed cortical potentials appear as discrete wave and muscle potentials as 'solid' spikes. A speed of 30 mm. per second is economical of paper as is consistent with good visual discrimination. The recording time is about half to one hour. When records are being taken, the important changes of wave pattern are marked out. At the end part of all the recording, the subject is asked to hyperventilate. This is helpful in detecting the convulsive disorder, if there is any, because in hyperventilation alkalinity of blood increases and causes a metabolic change which precipitates the seizure discharge. One case in our series showed such abnormality during and after hyperventilation. Each day, after the record is completed, a visual analysis is done. In the records a square-wave calibration is included at the start and at the end.

In expressing data the name 'alpha' is meant to cover the range of frequencies between $8\frac{1}{2}$ to 12 per second, 'beta' 15 to 50 per second,

'theta' suggested as a designation for waves with a frequency of four to seven per second, and 'delta' half, one to six per second. The delta voltages are generally higher and there may range from 60 to 1,000 microvolt, whereas beta 5 to 30 microvolts and alpha 50 to 100 or little more microvolts. With these data in hand all records are analysed.

A type of records, occasionally found in the series taken here, is of low voltage with little or no recognizable frequencies and also considered normal. In this type of record (LV=low voltage; LVF=low voltage fast) the few waves that can be counted are for the most part fast, but the voltage is below ten microvolts. This type of record becomes common in 13 years of age, when $10\frac{1}{2}$ per second electro-encephalograms have become usual. In adult population it is intermediate in point of commonness between the $10\frac{1}{2}$ and 11 per second types of EEG. It is, therefore, classified as a type of normal electro-encephalogram. When the activity is below eight and a half second which occurs in eight per cent of adult control subjects and 16 per cent of epileptics is classified as slightly a normal.

Out of the total 50 cases which have been completed, alpha rhythm of the following percentage has been found in the series :—

	Per cent.
Eight and a half to nine per second ...	2
Nine and a half to ten per second ...	34·9
$10\frac{1}{2}$ to 11 per second ...	37·2
$11\frac{1}{2}$ to 12 per cent ...	6·9

This value may not, however, be accepted as final unless a large number of records from different aspects, viz. climatic conditions, nutritional status and other modifying factors, such as age and sleep, are duly considered.

23. Inquiry on EEG Studies in Epilepsy under Dr. N. S. Vahia at the Seth G. S. Medical College, Bombay.

With the co-operation of our sister institution, the B. J. Wadia Hospital for Children, the electro-encephalograms of children of various age groups up to 12 years are being taken and an average record of each age group will be decided.

Selected cases of different types of epilepsy are being taken up for the electro-encephalographic study and then the electro-encephalographic and therapeutic progress will be studied and the relationship between electro-encephalographic changes and clinical results evaluated.

Statistical data is being collected from over 250 cases of epilepsy to study the incidence of normal tracing in definite cases of epilepsy; and also to study the type of abnormalities in different types of epilepsy.

So far 121 cases have been studied from that point of view. Each electro-encephalograph was classified as positive, if it was indicative of, or compatible with the clinical diagnosis of epilepsy and negative if it was normal or within the limits of normality.

Tabulated Data on 127 E.E.G. Tracings

Type of Epilepsy	No. of E.E.G. taken	No. Positive	No. Negative
Grand mal	89	56	33
Petit mal	7	4	3
Psychomotor	2	2	...
Myoclonic attacks	3	2	1
Jacksonian attacks	5	4	1
Grand mal <i>plus</i> Petit mal	10	6	4
Grand mal <i>plus</i> Psychomotor	3	3	...
Grand mal <i>plus</i> Jacksonian	1	...	1
Infantile convulsions	1	1	...
	121	78 (64.5%)	43 (35.5%)

24. Study on the ætiology of mycotic granulomas occurring in India under Dr. H. S. Andleigh, at the S. M. S. Medical College, Jaipur.

An attempt has been made to find out the nature of the different organisms which cause mycetoma foot. Altogether 30 cases clinically resembling mycetomas were studied. Out of these 21 turned out to be those of mycetoma foot. These included five cases due to the ærobic actinomyces and the remaining sixteen due to the various species of fungi. The diagnosis and the differentiation between the two types of mycetoma was based on histopathological examination. Cultural examination was not very successful due to the lack of special media. It was possible to grow the fungi only from two out of the sixteen cases on the routine media. It was not found difficult to differentiate between the actinomycotic mycetoma due to actinomyces and the maduro-mycotic mycetoma due to the various species of fungi by histological examination using the Gram's and the Hotchkiss-Mc-Manus staining methods. With the Hotchkiss-Mc-Manus technique the fungi took bright red stain, while the actinomyces did not take up the red stain. With the Gram's method the actinomyces were stained as distinctly Gram-positive rods and though the fungi were also stained Gram-positive, they did not stand out as prominently as with the Hotchkiss-Mc-Manus technique. Using these methods of staining, it was easy to recognize the actinomyces as the slender branching bacilliary forms about one micron in diameter, while the fungi were formed of larger mycelia and spores about four to six microns in diameter. Contrary to the general belief the cases of mycetoma due to the ærobic actinomyces were found to be involving the bones as readily as those due to the fungi, but the extent of the involvement of bones and the deformity produced was certainly less in the cases of mycetoma due to ærobic actinomyces than in those due to fungi. Out of the five cases of Actinomycotic mycetomas studied, there was an interesting case involving the thigh instead of the foot. The incidence of mycetomas due to fungus infections was found to be about seventy six per cent, while only about twenty-four per cent of all the mycetoma cases were due to actinomyces. It is interesting to note that

all the five cases were due to the ærobic type of actinomyces and none due to the anærobic species.

Out of all the mycotic granulomatous conditions occurring in India, mycetoma foot is of the greatest importance. It has not only the highest incidence but also invariably needs amputation of the foot in the stage in which the cases come to us these days. The ætiology as well as the treatment of the maduro-mycotic mycetomas occurring in India have been studied to only a very little extent. There are certainly several species of fungi causing mycetoma foot in India but their biologic and pathogenic characters do not seem to have been studied so far. It is also not known if the species of fungi causing mycetoma in India are sensitive to any chemotherapeutic agent.

25. Investigation of factors producing renal changes in hydronephrosis and their rôle in the pathogenesis of renal infections under Dr. V. S. Mangalik and Dr. R. M. L. Mehrotra at the King George's Medical College, Lucknow.

The frequency with which renal infections become super-added on obstructive lesions of the urinary tract has drawn the attention of experimental workers for sometime. Hydronephrosis constitutes one very important condition under the group of obstructive urinary lesions. Attempts have, therefore, been made to investigate the nature of pathological changes and their evolution in hydronephrosis and the reasons underlying the readiness with which infection localizes under these conditions.

Hydronephrosis was produced in rats and rabbits and structural changes in the renal parenchyma investigated.

Experimental procedures.—In all 50 adult, healthy rats of both sexes and weighing 100 g. to 150 g., and ten healthy rabbits weighing 1 kg. to 1.5 kg. have been used. The animals were kept on synthetic diet and given water *ad lib.* Under ether anæsthesia, abdomen was opened by a mid-line incision and the left ureter ligated about 2 cm. below its origin. The ureter was then severed in between two ligatures. The abdomen was then closed by suturing the abdominal muscles and the skin separately. All operative procedures were carried out under strict aseptic conditions. The animals after the operation were put in wire cages, and given food and water *ad lib.* The animals were sacrificed in batches of 2 to 4 at 1, 3, 5, 7, 9, 11, 23 and 28 days and 5, 6, 8, 11 and 30 weeks. The kidneys were weighed before and after the removal of the pelvic fluid which was collected for chemical and microscopical examinations. The organ was then cut longitudinally in some animals and transversely in others. Pieces of tissue were fixed in buffered ten per cent formalin and 80 per cent alcohol. Paraffin sections were cut at 4 U to 5 U in the usual fashion and stained with Harris hæmatoxylin and eosin, Wilder's method for reticulin fibres, iron hæmatoxylin and van Gieson stain and periodic acid Schiff technique for mucopolysaccharides. Histochemical methods for the determination of alkaline phosphatase in kidneys were carried out according to Gomori's technique. In some animals India ink was injected in the aorta before removing the kidneys and renal vasculature studied in the organ by examining frozen sections. In representative organs Oliver's

technique for studying the isolated nephrons was employed. The pelvic fluid was examined chemically for urea and microscopically for cellular contents.

RESULTS.

Gross changes in obstructed kidneys.—Twenty-four hours after ligation of the ureters, the kidneys appear slightly enlarged and swollen, but the ureter and the renal pelvis do not present marked abnormality. At three days, the renal pelvis and the ureter are slightly distended and contain clear yellow urine. By fifth day, the organ is considerably enlarged and the pelvis and the ureter markedly distended with clear fluid. Both the cortex and medulla show marked pallor on cut surface, the pallor being more evident in the medulla. The calyces are dilated. At seven, nine and eleven days these changes become pronounced. By 23rd and 28th days the kidney has a globular and lobulated appearance, the cortex is thin and the papilla flattened and small. The renal pelvis is filled with a slightly turbid fluid which contains leucocytes, epithelial cells and red blood cells. The capsule of the organ is thickened and shows fine adhesions with the adjacent peri-renal tissues. At five, six, eight and eleven weeks these changes are more advanced and the fluid in the renal pelvis now has a dark brown or red appearance. The kidney cortex is very thin and atrophic. By 30th week the cortex of the kidney appears as a thin transparent membrane and contains yellowish-brown fluid. The outer surface of the organ has a lobulated appearance.

The wet weights of the obstructed kidneys (after removal of fluid in the renal pelvis) were found to increase up to four weeks and after this period they decreased progressively. The rate of gain in weight during the initial stages of ureteric obstruction was variable and appeared to be due to interstitial oedema and accumulation of fluid in the dilated tubules. The pelvic fluid by three to four weeks is slightly turbid with a light-yellow coloration. At five, six, eight and eleven weeks it is, however, thicker in consistency and dark-red in colour due to the presence of large amounts of blood. It again clears slightly by 30th week.

MICROSCOPIC CHANGES IN OBSTRUCTED KIDNEY.

After 24 hours the obstructed kidney shows slight dilatation of distal convoluted and collecting tubules. The proximal convolutions show cloudy swelling. By third day the proximal convolutions are also distended and their lining cells appear cuboidal. The interstitial tissue is slightly oedematous and shows sparse infiltration with lymphocytes. At five, seven and eleven days the hydronephrotic changes are well evident. The Bowman space, in several glomeruli, is increased and the interstitial tissue of the kidney presents some fibroblastic proliferation. Many tubules are dilated while a few appear small and atrophic; their lining cells show cytolytic changes. At 23 and 28 days, a number of convoluted tubules have disappeared and replaced by young fibrous tissue. The glomeruli, however, seem to escape destruction and their tufts are filled with red blood cells. Some of the surviving tubules are markedly dilated while others are atrophic. The surface of the renal papilla is necrotic and congested (infarction). By 35 days, almost all the proximal convoluted tubules have disappeared, the interstitial fibrous tissue is markedly increased and contains many inflammatory cells. The collecting tubules and the distal

convolutions are atrophied. The number of dilated tubules which survive are few, glomeruli appear small in size and their capillary basement membranes are thickened. At 8, 11 and 30 weeks, the glomeruli are remarkably close together being separated only by collagen fibrils and occasional tubular remnants.

In the early stages, the changes in kidney seem to present a group distribution, the changes being more marked at some places and little or absent at others. As hydronephrosis progresses the atrophic changes become more or less diffuse and are most advanced laterally than in the central part of the renal medulla. The convoluted tubules disappear first, then the looped portions and the collecting portions of the tubules, while the glomeruli resist the longest. Later on, the number of glomeruli also becomes less and less and finally only a few of them are discernable at the periphery of the renal parenchyma which by now consists of connective tissue only.

Experiments are also in progress to study the localization of bacteria in the hydronephrotic kidneys and to investigate the probable mechanism for such localization.

26. Inquiry on infantile diarrhœa and vomiting under Dr. G. K. Tiagi at the S. N. Medical College, Agra.

Eighty-five cases of infantile diarrhœa have been investigated so far. Seventeen of these were from the maternity wards of S. N. Hospital, Agra, fifteen of not more than eight days old, one fifteen days and one of twenty days old. One infant of four months age was from the children ward and the remaining sixty-seven cases were from the Children and Female Outpatients' Departments of S. N. Hospital, Agra. Sixty-three infants of under one year and five under one and a half years age are included in this series of sixty-eight. No epidemic of infantile diarrhœa and vomiting has been noticed up to this time.

SOURCE OF MATERIAL.

Maternity ward											Children ward			Female & children out-patients department			Total	
17 cases											1 case			67 cases			85	
Days														Months				
Age	2	3	4	5	6	7	8	15	20	1-3	3-6	6-9	9-12	12-15	15-18			
Number of cases	1	1	7	3	...	2	1	1	1	10	23	11	19	1	4			
Duration of disease in days	...		1	2	...		3	4	5	...		1-4	5-8	9-12	13-16	17-20	20-30 & over 30	
Number of cases	...		5	5	...		1	2	4	...		32	10	12	6	3	4	1

Before rectal swabs were taken for culture, the severity of the illness, presence or absence of parenteral infection and dietary habits were noted. Most of these infants were taking mother's milk and diluted cow's milk. Few were also taking, in addition, pieces of bread. Most of these infants belonged to poor-class families. None of them indicated parenteral infection. Infants from maternity ward were either on mother's milk or on whey, and they were having loose motions without any blood or blood or mucus in them. Seven infants were having mucus and nine mucus and blood both and the rest sixty-nine were not having either blood or mucus. Vomiting was noted only in twelve infants and its frequency varied from three to eight times in 24 hours. Slight de-hydration was present only in eight cases.

Number of stools	Number of cases
Less than 5	6
6 to 10	55
11 to 15	15
16 to 20	9

None of the present series of cases showed any protozoal infection. Microscopically epithelial cells and leucocytes were present in all of them and red cells only in few. Bacterial flora was usually low.

Rectal swabs were cultured on MacConkey's plates, in the beginning one plate was used but later on two plates were used for each swab culture. Incubation of the plates was done aerobically. Type colonies were indentified and their proportion was noted.

RESULTS.

A. I. Twelve cases showed mostly the colonies which were non-lactose fermenters and these proved to be : (1) *Proteus vulgaris* in three cases, (2) *Proteus morgani* one case, (3) *Pseudomonas aeruginosa* in one case, and (4) *Paracolon* bacilli in seven cases, as confirmed by their biochemical reactions and pigment production.

II. Five cases showed preponderance of coagulase-positive *staphylococci* and two of these were from maternity ward cases four and seven days old infants.

III. Two cases showed *Streptococcus faecalis* and both of them were in maternity ward infants.

IV. One case showed *Micrococcus tetragena*.

B. The remaining cases gave only lactose-fermenting colonies.

I. Four of these produced mucoid, sticky colonies, proved to be *Aerobacter aerogenes*, by their biochemical reactions.

II. In sixty-one cases the smooth colonies as revealed by acriflavine were investigated from their biochemical properties. Acriflavine in one in 500 in normal saline agglutinates the rough strains.

Lactose, glucose, maltose, mannite, sucrose and dulcite were used for their fermentative reactions. Indole Production, Voges-Prausker's reaction,

methyl-red test, Koser's citrate-utilization test and formation of gas at 44° C., were also done.

One proved to be *Bact. coli communis*, forty-five *Bact. coli communior* and fifteen resembling *Aerobacter aerogenes*. All of these sixty-one cases were indole positive and gave positive methyl-red test. All of these also produced gas at 44° C. in lactose medium.

Method used for the preparation of Bact. coli 'O' serum.—Specific sera are now in the process of preparation from the two specific strains of *Bact. coli* D 433 and Aberdeen B which have been already obtained from the Central Public Health Laboratory at Collindale, London (England). The inoculum has been prepared by growing the organism overnight in 100 c.c. nutrient broth (inoculated with one loopful of a 24-hour nutrient broth culture). This 100 c.c. broth culture has been steamed for two and a half hours. Formalin has been added to a concentration of 0·3 per cent (i.e. adding 0·75 c.c. of 40 per cent commercial formalin to 100 c.c.). The suspension was kept at room temperature. The rabbit was given in five intravenous injections at four-day intervals with the following increasing quantity of inoculum : 0·25 c.c., 0·5 c.c., 0·5 c.c., 1·0 c.c., and 1·0 c.c. The rabbit was bled from the ear on the fifth and seventh days and bled out on the ninth day after the last injection. The pooled serum should give a titre of 1 : 6,400 to 1 : 12,800.

Method used for the preparation of Bact. coli 'OK' serum.—The inoculum was prepared by growing the organism overnight on thick 0·1 per cent glucose-agar plate. This plate was soft, containing one and a half per cent agar. A suspension of about 500 million per c.c. in one-fourth strength Ringer solution was employed as rabbit inoculum. (Fresh living suspensions had been made up not more than half hour before each inoculation). The rabbit was given five intravenous injections at four-day intervals with the following increasing quantities of inoculum ; 0·25 c.c., 0·5 c.c., 0·5 c.c., 1·0 c.c., and 1·0 c.c. The rabbit was bled from the ear on the fifth and seventh days and bled out on the ninth day after the last injection. This pooled serum should give a titre of 1 : 800 to 1 : 1,600. Sera giving a titre of less than 1 : 400 was discarded.

Sixty-one strains of *Bact. coli* isolated so far and those isolated later on will be tested with these specific sera by agglutination test.

27. Investigation on the contribution of social factors in the origin, continued prevalence and spread of so-called 'tropical diseases' under Dr. S. C. Seal and Professor K. K. Mathan at the All-India Institute of Hygiene & Public Health, Calcutta.

The first disease chosen in the present inquiry for studying the contribution of social factors in the continued prevalence of certain diseases in India was cholera. As ascertainment of relationship to social factors involves the assessment of other epidemiological factors which are intimately associated with social factors, an elaborate schedule had to be prepared for collecting information. This schedule was designed to collect information of the patients, relation to residential status, duration of stay in Calcutta, age, sex, marital status, religion, province of origin, occupation, diet, housing condition, type of locality, nature of water-supply, other sanitary facilities, economic condition, literacy, health knowledge and practices, addictions,

inoculation history, history of contact with cases, recent movements, type of medical treatment, preventive measures adopted, etc. The cases to be investigated were sampled from the list of patients attending the Nil Ratan Sarcar Hospital where the majority of the cholera cases of Calcutta were admitted and for whom fairly reliable diagnostic records were available. The field investigators visited the houses of the patients whose addresses were obtained from the above list, and collected information according to the schedule. A limited number of items of information included in the schedule relating to all the cholera deaths occurring in the city of Calcutta during the period under survey was also collected simultaneously from the records of the Corporation Health Department.

A total of 434 cases was investigated during the period under review. Though the time of study was too short to bring out the final conclusions, a few of the salient points noted in the course of the analysis of the data are given below :—

The wards which were severely affected by the cholera outbreak of 1953 were Entally, Tangra, Manicktolla, Jorabagan, Belgachia and Tollygunge. It was found that nearly one-fourth of the cholera patients studied were temporary residents of the city or were recent visitors. Comparing the percentage distribution of the province of origin of the patients with similar distribution for the residents of Calcutta, it was found that people hailing from Bihar were proportionately more affected than those of other provinces. Females were much more affected than males. Young age groups were also severely affected, 44 per cent of the patients being under 15 years of age though this age group constituted only about 25 per cent of the whole population. The communities which were mainly affected were Hindus and Muslims, the incidence being slightly higher among the Hindus. On the other hand, deaths were proportionately more among Muslims than Hindus, the ratio between number of deaths in the two communities being 80 : 18 compared to the ratio 83 : 12 giving their population strength in the city. The cases of cholera were mostly confined to certain occupational groups, viz. 56·2 per cent among those staying at home (including 25·7 per cent among the housewives) and 14·8 per cent among coolies, labourers and domestic servants.

More than two-thirds (67·8 per cent) of the cases were found in bustees and remembering that only five per cent of the dwelling units of Calcutta fall under the category of bustee, the concentration of cholera in bustee area is quite evident. About 90 per cent of the houses in which cholera cases were investigated, had more than one family and in 34 per cent of those houses more than eight families were living together. Eighty-nine per cent of the families investigated had less than 25 square feet per person. About 83 per cent of the families had no separate room for kitchen, one-third had no metal utensils, 80 per cent had no furniture and 15 per cent slept on floors.

The sanitary facilities of the houses were also very unsatisfactory. In nearly 45·7 per cent of the affected houses there were no arrangement for drinking-water supply and 93 per cent of the households procured water from street hydrants which contained Ganges water or from corporation water-carts. About 46 per cent of these households used obviously contaminated tank, river or canal water for domestic purposes. Only 5·6 per cent of patients, however, gave history of bathing in the Hooghly River or canal within one week prior to the attack of the disease. About half

the households had bucket latrines and 14 out of 434 families had no latrines at all. In nearly 60 per cent of the houses more than 25 persons had to use one latrine which in many instances were connected with drains and cesspools.

The educational standard of the patients was much below the average level of Calcutta as seen by the percentage of illiterate people in the group (85 per cent compared to 52 for Calcutta as a whole). As judged by the investigators, the outlook on food was either traditional or prejudiced in 42 per cent of the patients. Eighty per cent of the patients were rice-eaters, four per cent wheat-eaters and 14 per cent mixed rice-and-wheat-eaters. Seventy-seven per cent of the patients were eating fish and meat regularly. Thirty-six per cent of the patients were habituated to eating food from a common plate or to eating remnants left by others. Eighty per cent of the patients were known to have taken food cooked on previous day during the incubation period.

The economic condition of the patients was very poor as seen by the following facts : The per capita income of the family was less than Rs. 10 per month in 28 per cent, and less than Rs. 20 in 55 per cent ; 62 per cent of the patients had no personal income ; only 14 families out of 434 had their own houses, 16 per cent having no fixed residence at all ; 40 per cent of the patients were living in houses with rent less than Rs. 10 per month. The economic loss to families by deaths was as follows : In 20 per cent there was total loss of income, in 1·3 per cent three-fourth, in 6·6 per cent one-half, and in 3·6 per cent one-fourth of the total income.

The above findings based on the morbidity data were somewhat similar to the information obtained from the Corporation Health Department records on certain items relating to mortality data, for example, among 1,713 cholera deaths which occurred during the period under survey about 43 per cent occurred among children under 15 years ; the incidence was higher among females (48 per cent of deaths were among females who constituted only 36 per cent of the population) ; the condition of the privy or drainage was unsatisfactory in about 40 per cent of the houses and the same proportion had other sanitary defects ; about 63 per cent of the death were living in huts and 37 per cent only in *pucca* houses.

A critical study of these findings is in progress.

28. Bacteriological study of puerperal infections under Dr. Mohan Lal Sur at the Medical College, Amritsar.

SYNOPSIS.

Twenty-six members of the Medical College and Maternity Hospital staff were bacteriologically examined for listing of carriers. One hundred and thirty-one specimens were cultured—52 aerobically and 79 both aerobically and anaerobically. Fifteen of these members (66 per cent) were carriers of *Staph. pyogenes*.

Forty-eight pregnant women were bacteriologically examined for finding the carriers. Three hundred and seventy-seven specimens were cultured—144 aerobically and 233 both aerobically and anaerobically.

Five of them (about ten per cent) were carriers for *Staph. pyogenes* and one for *hemolytic streptococcus* group A.

Vaginal and cervical (external os) flora of 37 pregnant women was studied in detail. Cultures were put up both aerobically and anaerobically.

It was noticed that vaginal and os flora mostly showed only quantitative difference and not the qualitative difference.

Seventy-two hour cultures of seven cases yielded no growth. (It is presumed that these cases had some intravaginal antiseptics).

Bacterial flora of remaining 30 cases is as under :—

Staph. albus 22, *diphtheroids* 171, *micrococcus* 14, *Str. faecalis* 3, *anaerobic streptococcus* 9, *non-haemolytic streptococcus* 1, yeast 11, *lacto-bacillus acidophilus* 6, *Bact. coli* 1, *Str. haemolyticus* 0, *Staph. aureus* 0, *Cl. welchii* 0, (In one anaerobic culture a Gram-negative slender bacillus about 4μ . long with some filamentous forms had grown. Forty-eight-hour old colonies were pin-head size. It did not grow on sub-cultures. It is difficult to label this organism, but probably it belonged to the *fusiform* group).

Eight suspected cases of puerperal sepsis were investigated. At this stage it is not possible to make any statement as to the results achieved.

Bacteriology of the dust of labour rooms, lying-in rooms, and clothing (blankets) was investigated.

LINE OF WORK.

I. Bacteriological investigations of the cases.

1. *Antenatal*.—Routine procedure followed is swabbing and culturing from both hands, both nostrils, throat, vulva, vaginal fornices, any cervical discharge or erosion, and any septic focus on the body.

2. *During labour* (intra-partum).—If the ante-natal bacteriological investigations are not already done, the whole procedure except the vaginal fornices and the cervical cultures is gone through.

3. *Post-partum* : (24 hours, three days and seven days).—

(a) *Lying-in wards*.—(i) Lochia—swabs from the vagina. (ii) Blood cultures.

(b) *Delivered outside and admitted later*.

The whole procedure as under 1 and 3(a) is gone through.

II. Bacteriological investigations of the staff.

The undermentioned staff is being bacteriologically examined and records kept of any *haemolytic streptococci* or *Staph. pyogenes* isolated from their hands, throat, and nose :—

Surgeons, house surgeons, nurses and nurse dias.

III. Other investigations.

1. Bacteriology of the dust of labour rooms and lying-in rooms.
2. Bacteriology of the clothing, especially of the blankets used in maternity wards.
3. Check up of the hospital sterilizer used for dressing, etc.
4. Bacteriological study of any local septic lesion in the infant, especially the throat and the skin, and the breast of the mother.

The *Staph. pyogenes* strains isolated have been stocked for 'phage typing. The *hæmolytic streptococci* have been grouped. It is proposed to type them at a later stage.

At the completion of this work it will be possible to judge the rôle of the attendant carrier in the ætiology of puerperal sepsis by correlation of the *staphylococcal* 'phage types and the *streptococcal* types isolated from the patient on one hand, and the attendants on the other. By detailed investigation of the bacteriology of the ante-natal vagina and the bacteriology of the pureperal sepsis, some conclusions as to the rôle of the anærobic commensals in the ætiology of puerperal sepsis in these cases will be drawn.

29. Studies in mental health at Ramnagar (Closepet) under Dr. M. V. Govindaswamy at the Mental Hospital, Bangalore.

The area under study has a population of 73,865 comprising 133 villages besides two towns. There is a well-established Health Centre and Polyclinic at Ramanagaram. The doctors and ancillary personnel have intimate knowledge of the people living in the area. The population is mainly agricultural with silk-farming as a cottage industry, but it is not a typical rural unit. It is very near Bangalore, is the centre of considerable traffic, with a fluid population. The population has been subjected to a series of public-health and population studies. The area was selected only because of the facilities offered. The area is divided into five divisions comprising of villages and corresponding population as shown below:—

Division	Number of villages	Population
A	23	23,045
B	39	10,965
C	26	14,354
D	22	13,481
E	23	12,492

The investigation is limited to the study of: (1) Frank cases of psychoses, (2) evident psychoneurosis, (3) mental defect (idiocy and imbecility), (4) epilepsy and (5) drug addiction.

The first step was to find out what cases were regarded by the people of the community as presenting mental health problems. The village folk know very little about mental disorders, especially those with non-violent symptoms. They attribute abnormal behaviour of an individual to possession by evil spirits, witchcraft, poisoning by enemies, displeasure of demigods and so on. So to find out people with abnormal behaviour in the villages one has to speak to the people in their language. Initial contacts were made with nurses, patels, (village-headman) leading farmers and other influential persons, who in general extended their co-operation for this survey. Not much difficulty was experienced in spotting cases of major fits, as everyone in the village knew about them. Minor fits and other epileptic phenomena had to be explained in simple terms to find out such cases.

For case-recording the psychiatric case sheet used in the Mental Hospital, Bangalore, was followed. The symptoms of mental illness as described by the villagers were recorded. Particular attention was paid to the family history to get at the hereditary factors and a family geneo-logical tree was prepared for each case. The following factors were borne in mind during the inquiry:—

1. Factors inherent in the constitution of the individual : Hereditary factors including body-build.
2. Factors dependent on various age periods and epochs of life : Childhood, adolescence and puberty.
3. Sexual differences.
4. Exceptional stresses : Recent or remote, and in childhood.
5. Environmental and sociological influences : Industrial and rural.
6. Familial influences : Joint-family life, domestic troubles, marriage, etc.
7. Gross physical influences.
8. Nutritional state: Chronic debilitating diseases, like malaria, ankylostomiasis and associated anæmic conditions, virus diseases.

Work on the field was started on 1st July 1953. During the period under report (end of September 1953) 19 villages from 'B' division with a population of 7,787 and nine villages from 'A' division with a population of 2,401 have been visited and 66 cases have been met with. Tentative diagnosis were arrived at on the basis of case-histories and inter-views with the patients. Of the above cases 22 are frank cases of psychoses, seven of psychoneurosis, four mental defects, 31 epileptics and two drug addicts.

A. Psychoses		Number of cases
1. Schizophrenia	15
2. Paranoid state	1
3. Manic, depressive	3
4. Puerperal psychosis	3
B. Psychoneurosis		
1. Hysterical fits	1
2. Stammering	6
C. Mental defect		
D. Epilepsy		
1. Grand mal	20
2. Petil mal	6
3. Jacksonian epilepsy	1
4. Grand mal with mental defect		3
5. Psychomotor epilepsy	1

E. Drug addicts (Ganja)

Specimen of geneological tree.

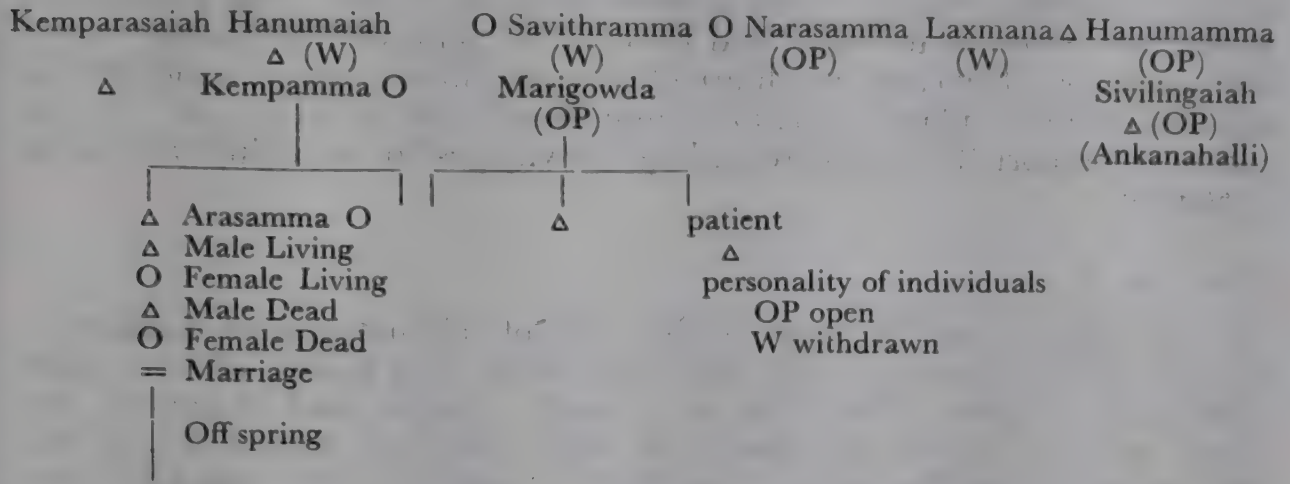
Village : Kailancha.

Kempe Gowda Δ = O Honnamma

Caste: Vokkaliga

(Kailancha) OP

OP (Voddarahalli)

*Epilepsy.*

Every case of epilepsy present, irrespective of the clinical type, presents a problem which can be approached, it is suggested, in the following way and by the following ten steps :—

- (1) The recognition of the epileptic nature of the condition.
- (2) The problem of neurological diagnosis. The recognition and location of focal (discharge if present). The exclusion of neighbourhood lesion (tumour, trauma, cysticercosis, cyst, etc.). The verification of the essential anatomical and functional integrity of the central nervous system.
- (3) The review of the genetic and constitutional factors.
- (4) The inquiry for physiological and biochemical precipitants (reflex sensory factors, influence of sleep, menstruation, time of day, diet, digestion and excretion, habits, occupation, etc.).
- (5) The inquiry for emotional factors (frustration more significant than anxiety or fear, influence of emotions of diet, breathing, sleep, weight, autonomic functions, libido).
- (6) The mental status ; personality and intelligence.

We may then proceed to consider :—

- (7) Removal or suppression of the focal epileptogenic area : possible surgery.
- (8) A plan for reducing the influence of physiological, biochemical and emotional precipitants.

(9) Suitable anticonvulsant therapy and the planning of a course of such treatment under observation, preferably with EEG control.

(10) Such measures as may be necessary to reduce to a minimum the effects of the epilepsy upon the patient himself and upon his environment (Denis Hill).

The investigations carried out during the last ten weeks (from 1st July, 1953 to the middle of September 1953) have been chiefly of a fact-finding nature and of establishing contacts to help subsequent investigations. On the work done so far the incidence of mental morbidity excluding epilepsy has been roughly of the order of three per 1,000. The conclusions drawn are of course tentative and have to be confirmed by further study. Summaries of a few cases which are of scientific importance or which throw light on how the villagers look at mental illnesses are given below. At the end of the report the details regarding the villages so far visited are given (*Appendix*).

Serial No. 1.

Byamma, age 20 years, is the wife of Thimmayya of Kailancha village. She delivered a child nine days back. No history of fever after child-birth is given. She talks irrelevantly, laughs and shouts for no reason and used to say her dead aunt had come ; and sometimes felt her dead aunt twisting her neck. The family history revealed that her elder sister, was behaving in the same fashion six months back. On physical examination the patient was found to be anæmic. She is of asthanic build, at the time of interview she was depressed and complained of visual and auditory hallucinations. The tentative diagnosis of puerperal psychosis is arrived at.

Serial No. 5.

Chikkaveeregowda of Ankanahalli village, age, 35 years, is married. Since three years periodically he beats and abuses his wife and parents for no reason. There is no family history of mental illness. In the interview with the patient he said that a friend of his had done witchcraft on him and poisoned him four years back. Since that time he feels stones falling on his head, intense pain in his fingers and sees his limbs separating from his body and dancing before him. He feels compelled to assault his parents and wife. A tentative diagnosis of paranoid condition was made.

Serial No. 6.

Thimmakka, age 35 years, is the wife of Karigowda of Voddarahalli. She is said to become mute once in a month or two, tries to run away from the house, and refuses food and remains without food for three to four days. This has been so for the last eight years. The family history reveals that the patient's elder sister committed suicide by hanging and she was supposed to be possessed by a ghost. Patient's another sister who died seven years back had a wandering tendency and used to talk irrelevantly. During the interview the patient was not co-operative, was depressed and told she sees the figures of dead people and hears their voices threatening to kill her. The family history of mental illness is very suggestive. A tentative diagnosis of schizophrenia was arrived at.

Serial No. 14.

Ningamma, age 45 years, married, is the daughter of Sangaiah of Madapura village. For the last ten years she has not been normal. She is all the time happy and laughing. She is restless and moves from place to place. Sometimes she is abusive. Her talk is irrelevant as "she speaks of something this moment and something unconnected the next". At times she does not speak at all and looks depressed. She washes herself five to six times daily. Family history revealed that the father of the patient was mentally ill as he used to spend days away from home in jungle. He was abusive and assaultive. Every year he used to be normal for three to four months. During the interview and examination the patient is found to be clean and well dressed. She looks happy and it is difficult to hold her attention. She does not answer questions. She talks to herself and there is flight of ideas. A tentative diagnosis of manic depressive state is arrived at.

Serial No. 10.

Siddalingamma, age 15 years, is the wife of Eswaraiah of Bannikuppa village. She is having fits since the last four months. Fits come once in three to four days. There are a series of violent jerking movements of the limbs. Three to four people have to control her. There is no loss of consciousness or frothy saliva from the mouth. Fits come always at night when all the people are at home. Personal history of the patient disclosed that there was misunderstanding between the patient's father and her husband about four months back and that she was not allowed to visit her parents. She started getting fits thereafter. She has had two abortions. First in the fifth month and the next in five and a half months pregnancy. At the examination of the patient physically nothing abnormal was found, and the patient complained of headache all over the head after the fit. There was no history of aura. A diagnosis of hysterical fits is proposed.

Series No. 3.

Nanjappa, age 30 years, single, belongs to Koongal village. He has no idea of his property and belongings. He is considered a fool by the people. He is a voracious eater. Personal history revealed that he was put to school but could not learn anything. He takes buffaloes for grazing to the fields. On examination it was found that the patient's head was large, face asymmetrical and that he had a squint. His mouth is large and tongue is small. He gave his name, names of his parents and the name of the village. He cannot do simple arithmetic. He can protect himself against physical dangers. A diagnosis of mental defect is suggested.

Serial No. 14.

Basappa, age 30 years, belongs to Bibhuthikere village. The patient is behaving abnormally since the age of two years, in that he gives a cry all of a sudden and says he is seeing the figure of an old woman, and that he is terrified. Then he asks the people round about to abuse the figure and gets under a blanket himself. He also says that he gets terrible discomfort in the epigastric region prior to each such episode. This occurs once in 15 to 20 days. There is no loss of consciousness. On examination

nothing abnormal physically or mentally was found. Provisional diagnosis of psychomotor epilepsy was made.

Serial No. 16.

Channa, age 16 years, is the son of Eregowda of Hunasanahalli. The patient has transient loss of consciousness off and on since eight years. Previously, this was happening once in 10 to 15 days. Since one and half months he is having such attacks daily. The face of the patient becomes blank all of a sudden while he is eating or walking about. He drops things he is holding and falls down unconscious. After such an attack he eats grass and cowdung. Previous history revealed that the patient had an attack of paralysis on the left side of the body at the age of two years and that he was not able to walk till the age of eight years. Personal history showed that he was dull, did not know common things and that he was not sex-conscious and would walk naked in the presence of women. Previously he was taking cattle for grazing but since one month he was not doing any work. On examination face is assymetrical, mouth is large and the tongue is small. He has a squint. There are signs of old remiplegia on the left side. He was not able to give his name, names of his parents and name of the village. He recognizes coins as such but does not know their value. He cannot do simple arithmetic. He protects himself against physical dangers. The case is grand mal with mental deficiency.

Serial No. 20.

Ningappa, age 23 years, is married and belongs to Rangarayaradoddi village. He complains of loss of consciousness daily seven to eight times. As he was talking, walking or eating the face becomes blank, and eye-balls are rolled up. He does not answer questions. After a minute he regains consciousness and continues what he was doing. Family history revealed that his child aged nine months was having attacks of major fits for the last one and a half months. The case with father having petil mal and his child grand mal is interesting.

APPENDIX.

Serial Number	Name of the village	Male	Female	Total
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' A ' division.

1.	Channamana halli ...	273	285	558
2.	Rangarayara doddi ...	104	106	210
3.	Basavanapura ...	180	169	349
4.	Voddara doddi ...	51	50	101
5.	Sangabasavana doddi ...	189	158	347
6.	Archikarahalli ...	205	69	274
7.	Sivanegowdana doddi ...	124	118	242
8.	Madapura ...	129	143	272
9.	Kumbapura ...	78	74	152

' B ' division.

1.	Achalu ...	109	104	213
2.	Krishnapura doddi ...	174	170	344
3.	Gowdaiahna doddi ...	132	123	255
4.	Averahalli ...	378	349	727
5.	Kailancha ...	305	211	516
6.	Kotahalli ...	208	154	362
7.	Hunasahalli ...	687	582	1,269
8.	Koonagal ...	168	177	345
9.	Moodlahalli ...	57	44	101
10.	Naguhalli ...	108	103	211
11.	Hulikere ...	173	154	327
12.	Gunnur ...	268	243	511
13.	Voddarahalli ...	206	167	373
14.	Chikkenahalli ...	83	76	159
15.	Kaveri doddi ...	65	74	139
16.	Vibhuthikere ...	240	185	425
17.	Bannikuppa ...	558	507	1,065
18.	Ankanahalli ...	180	163	343
19.	Achalu doddi ...	45	57	102

SUMMARY AND CONCLUSIONS.

The report summarizes the investigations on mental morbidity in Ramnagar near Bangalore from 15-6-53 to 30-9-53 in 133 villages, covering a population of 73,865. The period is too short to draw any definite conclusions, but the following are suggestive. The samples are too few for any sociological or statistical analysis :—

1. The incidence of epilepsy seems to be in the order of 1 in 250. It is a very high figure and has to be verified on a larger population. EEG cardiozol and insulin sensitivity on the relatives of such patients are proposed to be studied.

2. The incidence of mental morbidity of easily identifiable and long-standing psychoses and related illnesses seems to be in the order of 3 per 1,000. This is the minimum. From a general practitioner's point of view, mental disorders fall into four categories :

- (a) Cases which the general practitioner can treat actively and rationally himself (50 per cent).
- (b) Cases which are suffering from a self-limiting illness, and which need supervision until a natural remission sets in (25 per cent).
- (c) Cases which require expert help and advice and must be referred to a psychiatrist (ten per cent).
- (d) Cases which are chronic and untreatable by any known methods (15 per cent). Most of the cases reported seem to belong to the fourth group, and hence the incidence is very much greater than the 3 per 1,000 of the population mentioned.

3. This is a matter for verification, a larger survey and statistical and psychological analysis.

4. Malaria is not a significant factor in influencing these illnesses in this area. Anæmias, however, seem to play a part both in ætiology and in the clinical picture.

5. Since this is not a large industrial area, problems of industrial neuroses, delinquency and of social pathology are not very apparent. More intensive survey and investigation is necessary.

6. This is only a pilot study but seems to be full of promise.

30. Studies on the false reaction in flocculation test for sero-diagnosis of syphilis under Dr. A. K. Mitra, V. D. research worker, Government of West Bengal, Calcutta.

In the preliminary report it was shown that if syphilitic sera after dilution to a convenient volume (1 in 16) with 0.9 per cent saline be subjected to fractional precipitation with varying concentrations (one-third and half saturation) of ammonium sulphate, bathed at a temperature of 56°C. for 15 to 20 minutes, and precipitates (ppts.) finally collected after high-speed centrifugation, then :—

- (i) The total globulin and pseudoglobulin ppts of all syphilitic sera showed reagin activity at a much higher dilution than that obtained with the corresponding whole sera.

- (ii) The reagin activity was observed at least in one in eight dilutions with the total globulin and pseudoglobulin precipitates, even though the corresponding whole sera showed reagin activity only up to two dilutions or less.
- (iii) The euglobulin precipitated by the above method (one third saturation) did not show reagin activity in most of the cases of low-titre sera. Only the euglobulin of high-titre sera showed reagin activity in a much lower dilution than the corresponding whole sera.
- (iv) No reagin activity was observed with the total globulin and pseudoglobulin precipitates of the biologically false reacting sera, at the eight-dilution level, although the corresponding whole sera did show a positive reaction up to eight dilutions or more.

Based on the above experimental evidences a technique was developed whereby the total globulin is precipitated from 0.03 c.c. of serum (diluted to 1 in 16) by half saturation with ammonium sulphate, and the precipitate tested for reagin activity with standard Kahn and V.D.R.L. cardiolipin antigen, starting from the minimum level of eight dilutions upwards. The technique was named as 'globulin test'. A number of biological false-positive reactions were detected in leprosy and malaria by adopting this technique, of which numbers and statistical data have been shown in the preliminary report.

Subsequent to the preliminary work as mentioned above 2,406 sera have been tested by the following methods :—

Present work	Standard Kahn test, V.D.R.L. slide test, Microkahn or slide modification of standard Kahn test, and the Globulin test (cf. preliminary report) with Standard Kahn and V.D.R.L. cardiolipin antigen. Sero- logical follow-up and clinical evaluation have been completed in some of the cases. The results are shown in Table I to III.
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The sera were obtained from the following group of cases :—

*(A) Fifteen diagnosed cases of *Lepromatous* leprosy with no clinical evidence of syphilis that have already been detected seropositive by one or more standard tests and then followed up on an average to more than nine months.

*(B) Cases of group A tested about one and a half month after the completion of treatment with six mega units of P.A.M. spread over ten bi-weekly injections.

(C) Nineteen hundred random samples from the ante-natal clinics of Lady Dufferin Victoria Hospital and Eden Hospital, Medical College, Calcutta.

(D) One hundred and sixty-one cases, referred from V. D. clinics, Sambhunath Pandit Hospital and Medical College Hospitals. The histories of these patients were not known before testing their sera.

*These were simultaneously tested by the Department of Serology, School of Tropical Medicine, Calcutta, by the Kahn's universal serological method. The results have not yet reached us.

(E) Three hundred and thirty diagnosed syphilis cases, before, during, and after treatment.

Only the positive results and the disagreement in results amongst different tests are shown in Tables I to III. Results of A and B are shown in Table I and those of C and D in Tables II and III, respectively. Cases which gave no reaction in any of the above tests have got no bearing with false reaction ; hence they have been omitted from the result table. Results of pregnant women who have a bad obstertical history suggestive of syphilitic infection, but seronegative, have not been shown, as their other investigations are not yet complete.

Results of E have not been tabulated but certain significant points have been observed in this group. They are :—

- (1) The globulin test with V.D.R.L. cardiolipin antigen is a more reliable guide to the clinical condition of the patient than the same with standard Kahn antigen.
- (2) The lower the titre in the standard Kahn or V.D.R.L. slide test, the greater is the titre difference with the corresponding globulin tests.
- (3) In high-titre sera, the titre difference is sometimes nil.
- (4) In an effectively treated case of syphilis the globulin test almost always becomes negative earlier than the corresponding standard test. From a study of the character of the ppts. under the microscope, it is often possible to predict whether the serum is obtained from a treated case of syphilis.

If the treatment has not been effective, the globulin test does not become negative even if the whole serum show a positive reaction at a very low titre.

It was expected that the globulin test would reveal a considerable number of false-negative reactions in pregnant women suspected of having syphilis ; but contrary to the expectation only two cases so far have been detected in this group to be positive in the globulin test and negative in all other tests. Of these one has been followed up and confirmed as syphilitic and the other still remains to be followed up. Clinicians, however, are of opinion that there certainly exist quite a number of pregnant women whose repeated miscarriages cannot be attributed to any cause other than syphilis, though they are seronegative. On inquiry into the problem from the literature it is found that there is a definite relationship between the cholesteral content of the blood and syphilis in pregnancy. Cholestrol (ester) is supposed to be at a lower level by about eight to eleven per cent in pregnant women with syphilis than in those having no syphilis. Besides, it has also been observed that the degree of cholesterolaemia in pregnancy is inversely proportional to the intensity of the serological reaction and vice versa.

Preliminary experimental works in this Laboratory indicate that this relationship between the reagin and lipids demand more attention than it has hitherto received. In fact, the revelation of this relationship between the lipids and the reagin will lead to a greater understanding of the serological problem of syphilis.

Besides the works already mentioned, a newer approach was made to determine the 'antigen-antibody' nature or otherwise of the floccules

of the standard Kahn-tested materials. It was reasoned that if the floccules of all the Kahn-tested materials be true 'antigen-antibody' floccules then they must fix complement, whereas any flocculation due to other causes will not fix complement unless the sera themselves are anti-complimentary. Based on this hypothesis, Kahn-flocculated materials from 250 sera were tested for complement-fixing activity. It was observed that :

(a) Most of the Kahn-positive floccules collected from all the three tubes of Kahn test showed strong C. F. activity, whereas materials from negative sera did not show any C. F. activity.

(b) Floccules from very weak or doubtful Kahn-reacting sera, e.g.

Kahn results.

1st tube	2nd tube	3rd tube
—	—	++
—	—	+
++	+	+
	—	+

showed no C.F. activity particularly in materials from the last tube unless the serum was from a syphilitic individual. Further observation in this line is necessary to come to any conclusion.

TABLE I.

Showing number of positive reactions obtained in lepromatous leprosy cases with different tests before and after treatment with P.A.M.

Name of tests	Number positive	Number followed up after penicillin treatment with their results	Remarks
All tests ...	4	1-Positive in all the tests. Titre remaining same in all tests except in V.D.R.L. and globulin test with V.D.R.L. antigen where the titre was increased by two and one dilutions, respectively.	Blood was taken while the patient was having a lepra reaction (? Anamnestic reaction).
V.D.R.L. and standard Kahn test only.	2	1-Positive in V.D.R.L. and Kahn test; titre remaining same.	? false reaction.
Standard Kahn test only.	6	1- { Negative in all tests. 2- { 2 Positive in Kahn test—titre increased by dilution	It was positive with Kahn test only in 1/2 dilution.

Three cases showed negative reaction in all the tests. Two of them were followed :—

- (1) Remained negative after treatment.
- (2) Showed positive reaction in standard Kahn test with neat serum after treatment.

TABLE II.

Showing number of positive reactions obtained in ante-natal cases with different tests.

Names of tests	Number positive	Number subsequently followed up with their results	Number remaining un-followed
All tests ...	43	11—All positive on subsequent follow-up. History strongly suggestive of syphilis.	32
All tests except V.D.R.L. slide-flocculative test	2	1—Positive in all tests on subsequent follow-up. History strongly suggestive of syphilis.	1
All tests except globulin test	3	1—Negative in all tests on subsequent follow-up. History does not point to syphilitic infection.	2
All tests except globulin test which was not performed standard Kahn test only	4 6	1—Negative in all tests on subsequent follow-up. History does not point to syphilitic infection.	4 5
V.D.R.L. slide test only ...	12	1—Negative in all tests on subsequent follow-up. History does not point to syphilitic infection.	11
All tests except standard Kahn test	1		1
Globulin test only ...	2	1—Positive in all tests except standard Kahn test on subsequent follow-up. Patient delivered a dead foetus. History also strongly suggestive of syphilis.	1
V.D.R.L. slide test and globulin test with V.D.R.L. cardiolipin antigen ...	3		3
Globulin test with V.D.R.L. cardiolipin antigen only ...	2		2
Globulin test with standard Kahn antigen only ...	1		1
Standard Kahn test and globulin test with standard Kahn antigen only ...	1		1
Totals ...	80	16	64

TABLE III.

Showing positive results obtained in the sera referred from V. D. clinics with different tests.

Name of test	Number positive	Number followed up and their results	Number not yet followed up
All tests	43	43—All cases of syphilis.	<i>Nil</i>
Standard Kahn T. & R.L. (globulin test V.D. negative)	7	4—(a) Treated syphilis—all test turned negative on subsequent test. (b) No evidence of syphilis—all tests turned negative on subsequent test (c) Patient with cancerous glands undergoing deep X-ray therapy. Standard Kahn test was negative but V.D.R.L. remained positive to 8 dilutions on subsequent test. 4—Granulomatous ulcer in pharynx and buccal mucous membrane. Biopsy examination shows non-specific chronic inflammation. No improvement with anti-syphilitic treatment.	3
All tests except standard Kahn test	1	1—Confirmed case of syphilis.	<i>Nil</i>
Globulin test with standard Kahn and V.D.R.L. cardiolipin antigen.	4	4—(a) Case of aneurysm of the aorta. Subsequent test showed the same result. (b) A case of gummatous ulcer of the skin, cured by anti-syphilitic treatment ; on subsequent test V.D.R.L. slide test also became weakly positive (c) A case of secondary syphilis. Serum showed extreme prozone reaction. (d) A case of secondary syphilis as in (c).	<i>Nil</i>
Globulin test with V. D.R.L. cardiolipin antigen only.	-1		1
V.D.R.L. and globulin test with V.D.R.L. cardiolipin antigen.	-1	1—All turned negative on subsequent test. Treated case of syphilis.	
V.D.R.L. slide test only	-10	6—Four cases of treated syphilis Two cases showed no evidence of syphilis. All turned negative on subsequent testing.	4
Totals	67	59	8

31. Epidemiological research in tuberculosis under Dr. P. V. Benjamin, Adviser in Tuberculosis, Director-General of Health Services, New Delhi.

The purpose of the present study was to investigate whether the level of tuberculin allergy induced in human beings by BCG vaccination is affected by exposing the vaccination site to strong sunlight immediately after the vaccine has been injected.

The level of tuberculin allergy has been disappointingly low in a great many of the groups of school children re-tested after mass BCG campaigns in some of the tropical countries. Studies directed toward finding the responsible factor (or factors) showed that vaccine exposed to direct sunlight or strong daylight, even for a matter of minutes, rapidly loses its allergy-producing property. The use of light-exposed vaccine undoubtedly accounts to a large extent for low post-vaccination allergy, but it does not appear to be the only cause.

In seeking other technical variations likely to occur under ordinary field conditions, sun-exposure of the vaccination site immediately after injection of the vaccine had been considered. Sunlight might affect the viability of the BCG organisms deposited in the *superficial* layers of the skin, and so affect the post-vaccination allergy. A preliminary trial was made in Denmark in 1951 by exposing a small group of children to a maximal dose of carbon-arc light within one hour of vaccination and at three-day intervals thereafter for a total of six weeks, while a comparable group vaccinated at the same time was not so exposed (Heinild *et al.*, 1952, *Acta Paediatrica*, **41**, pp. 415-436). The level of tuberculin allergy (and the average size of the local vaccination lesions) were not significantly different in the two groups when examined after six weeks. Another preliminary pilot-type study was made early in 1952 by Dr. M. L. Mehrotra, Medical Officer in-charge of the BCG teams in Kanpur, by exposing the vaccination site of a few children to direct sunlight for 30 minutes after vaccination and not exposing others (personal communication). Comparison of the tuberculin allergy in the two groups after eight months showed suggestive, but not significant, differences.

PLAN OF STUDY.

The present study was planned as a joint undertaking of the World Health Organization through the W.H.O. Tuberculosis Research Office and the Indian Government through the Indian Council for Medical Research. The work was done under the technical direction of the T.R.O. who provided a field team of two specially trained nurses, the salary for one team secretary recruited locally, field equipment and supplies, and was responsible for analysis of the results. The Indian Government assigned a doctor to the project, made arrangements for suitable facilities, and provided a secretary and a driver for the team. The Government and the Indian Council of Medical Research representatives acted as advisers for the project.

The work, carried out in 11 schools in Jabalpur during March and April 1953, comprised a total of about 2,000 children tuberculin-tested, 1,200 of whom were vaccinated and 700 re-examined five to six weeks later,

On the team's first visit to the schools, all children were given an intradermal (Mantoux) test with 5 TU (0.0001 mg.) of PPD, batch RT XIX-XX-XXI from Statens Seruminstitut, Copenhagen. Three days later the reactions were read by measuring the transverse diameter of erythema and induration with a mm. ruler. Children with indurations measuring less than 5 mm. were considered eligible for BCG vaccination and were allocated, by rotation, to three groups. Group A was to have the site of vaccination exposed to direct sunlight immediately after the vaccine had been injected; group B was to have the site exposed after a varying interval of time; group C was to have no exposure during the study period. Before vaccination, the clothing was removed from the child's left shoulder and he was given a special mark to identify the group to which he had been allocated. Vaccination was then given by injecting 0.1 c.c. of BCG vaccine, Statens Seruminstitut batch 1,038 or 1,039.

Immediately after vaccination each child in group A was sent outdoors into the strong, direct mid-day sunshine to expose his vaccination site. He stayed in the sunshine during the remainder of the 45-minute period required to finish vaccinating all of the children. Children in group A thus had their vaccination sites exposed for periods ranging from 0 to 45 minutes, the exposure in each child beginning immediately after he had been vaccinated. They were then brought indoors for a full 45-minute period.

Children in group B were kept indoors during the 45-minute vaccinating period, then they were sent outdoors into the sunshine to expose their vaccination sites for 45 minutes. Thus, all those in group B had a full 45-minute exposure period, but it began from 0 to 45 minutes after vaccination.

Group C were kept indoors during the two successive 45-minute periods.

Though the study design may seem rather complicated, its purpose was to be able to make a quantitative evaluation of the effect of immediate or delayed exposure to sunlight, if exposing the vaccination site affects the development of tuberculin allergy or the local vaccination lesions.

The team returned to the schools five to six weeks later to examine the vaccinated children. They were tested again with Mantoux 5 TU and the lesions at the vaccination site were measured and described. New students in the schools and students who had been absent for the pre-vaccination examination were also tested with 5 TU at this time.

Throughout the study, efforts were made to have the work as uniform and objective as possible. Details of how the work was to be done were written in advance and each person on the team was responsible for a particular job. For both the pre- and post-vaccination examinations, one nurse gave all the tuberculin tests and another read all the reactions. The reader dictated her measurements to a secretary so she would not see the child's card; thus, the reader could not be influenced by knowing the size of a child's previous reaction or even whether he had been vaccinated, as new students were included with the study group during the post-vaccination examination.

RESULTS.

Frequency distributions by size of induration of 5 TU tuberculin reactions are given in Fig. 1 for each of the three groups of vaccinated children. The results are remarkably alike. Children whose vaccination

sites were immediately exposed to direct sunlight (group A) and those whose exposure was delayed (group B) developed the same average degree of allergy as those with no exposure (group C). The mean reaction size was 13.5 mm. for all three groups, with the same spread of reactions (standard deviation) around the average.

The line diagrams in Fig. 2 show the mean size of tuberculin reactions and local vaccination lesions according to duration of sun-exposure immediately after vaccination (group A, upper fig.) and according to the time interval between vaccination and the 45-minute exposure period (group B, lower fig.). Neither duration of exposure nor the interval before exposure appears to influence the results: for both allergy and lesions, the curves show only minor and inconsistent deviations from the corresponding mean values for the non-exposed controls (group C).

It thus appears that immediate or delayed exposure of the vaccination site to direct sunlight, for periods ranging up to 45 minutes, has no significant effect on the degree of tuberculin allergy or the size of the local lesions present after five to six weeks.

In addition to the results germane to the study's main purpose, the pre-vaccination tuberculin tests provided information on the pattern of naturally acquired tuberculin sensitivity in Jabalpur school children. The upper section of Fig. 3 gives frequency distribution by size of reactions to the 5 TU test for nearly 2,000 children. The distribution is clearly divided into two concentrations of reactions, one group (on the left) with little or no reaction, and the other (on the right) with strong reactions. And, as sensitivity to tuberculin is taken as a sign of infection with tubercle bacilli, it is reasonable to assume that most of the children with strong reactions—the group centring around 16 to 17 mm. on the scale—represent the infected part of the population, the positives; and those on the left-hand end of the scale are the uninfected.

Separating the children according to age gives the frequency curves shown in the middle section of Fig. 3. As would be expected, the younger age groups contain relatively more negatives and correspondingly fewer positives than the older ones. There is a transfer with age, from the negative to the positive range on the scale, with a persistently low frequency of reactions in the area between the two groups. This, too, is consistent with the assumption that the children with strong reactions have been infected.

Further information is gained from the results of testing all of the patients in three tuberculosis hospitals: Lady Linlithgow Sanatorium, Kasauli; King Edward Sanatorium, Dharampore; and Lady Hardinge Sanatorium, Dharampore. The frequency distribution by size of 5 TU reactions (lower section of Fig. 3) shows a single group concentrated around an average of nearly 17 mm. Some have very large reactions, some small, but the group as a whole has reactions of about the same average size and covering about the same range on the size scale as the presumably infected children. Both groups center around 16 mm. to 17 mm. and all but a very small proportion have reactions of from 8 mm. to 24 mm. In this connection, it may be noted that practically all of the patients (99 per cent of them) had reactions measuring at least 5 mm. in diameter to the 5 TU test.

In Fig. 4 comparison is made between the allergy found in children who had not been previously tested (upper section) and in children vaccinated

five to six weeks earlier (lower section), both groups being tested at the same time, by the same person. Contrast is seen between the forms of the two distributions. The unvaccinated children, as already seen in Fig. 3, are clearly separated into two groups—the presumably infected on the right and the uninfected on the left. But the vaccinated (like the tuberculosis patients) form a single group whose level of allergy may be characterized by the average reaction size, 13.5 mm. of induration. Though the allergy found in school children after BCG vaccination was somewhat lower than that of the naturally infected, it may be that the maximum level of allergy had not yet been attained as the re-testing was done so soon after vaccination.

SUMMARY.

Nearly 2,000 school children in Jabalpur were tuberculin tested, and 1,200 of them vaccinated, early in 1953 in a co-operative study designed to determine whether exposing the vaccination site to strong sunlight immediately after injection of BCG affects the development of tuberculin allergy. Follow-up examination in five to six weeks showed that the tuberculin reactions (and vaccination lesions) averaged the same size in three groups of vaccinated children: (a) those whose vaccination site was exposed to sunlight for a period ranging from 0 to 45 minutes immediately after vaccination, (b) those exposed for a full 45 minutes after an interval ranging from 0 to 45 minutes after vaccination, and (c) those not exposed.

The pattern of tuberculin sensitivity in unvaccinated school children showed the presence of two groups fairly well separated by the 5 TU test: those with little or no reaction (the negatives) and those with fairly strong reactions (the positives). Patients hospitalized for tuberculosis were found to have about the same level of allergy as the positive (presumably infected) school children, and children vaccinated with BCG had a somewhat lower level of allergy when retested five to six weeks after vaccination.

The results of this study suggest that no precautions need be taken in practical BCG programmes to shield the vaccination site from sunlight after the vaccine has been injected. Furthermore, there is no indication that high levels of allergy cannot be produced by BCG vaccination of Indian children: the allergy five to six weeks after vaccination was almost as high as that found in the natural positives.

FIG. 1.

Distribution by size of induration of Mantoux 5 TU reactions 5-6 weeks after vaccination.

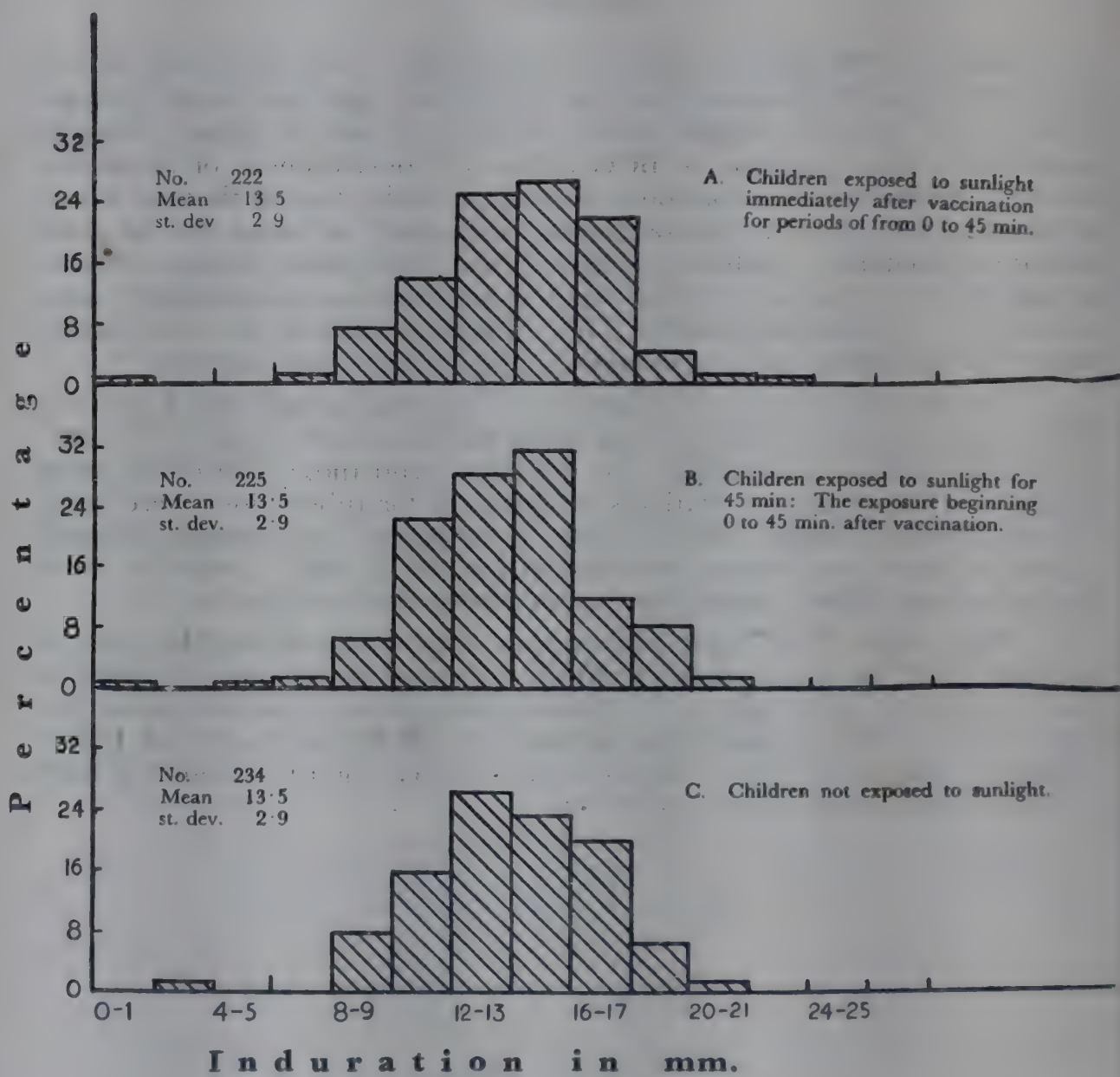
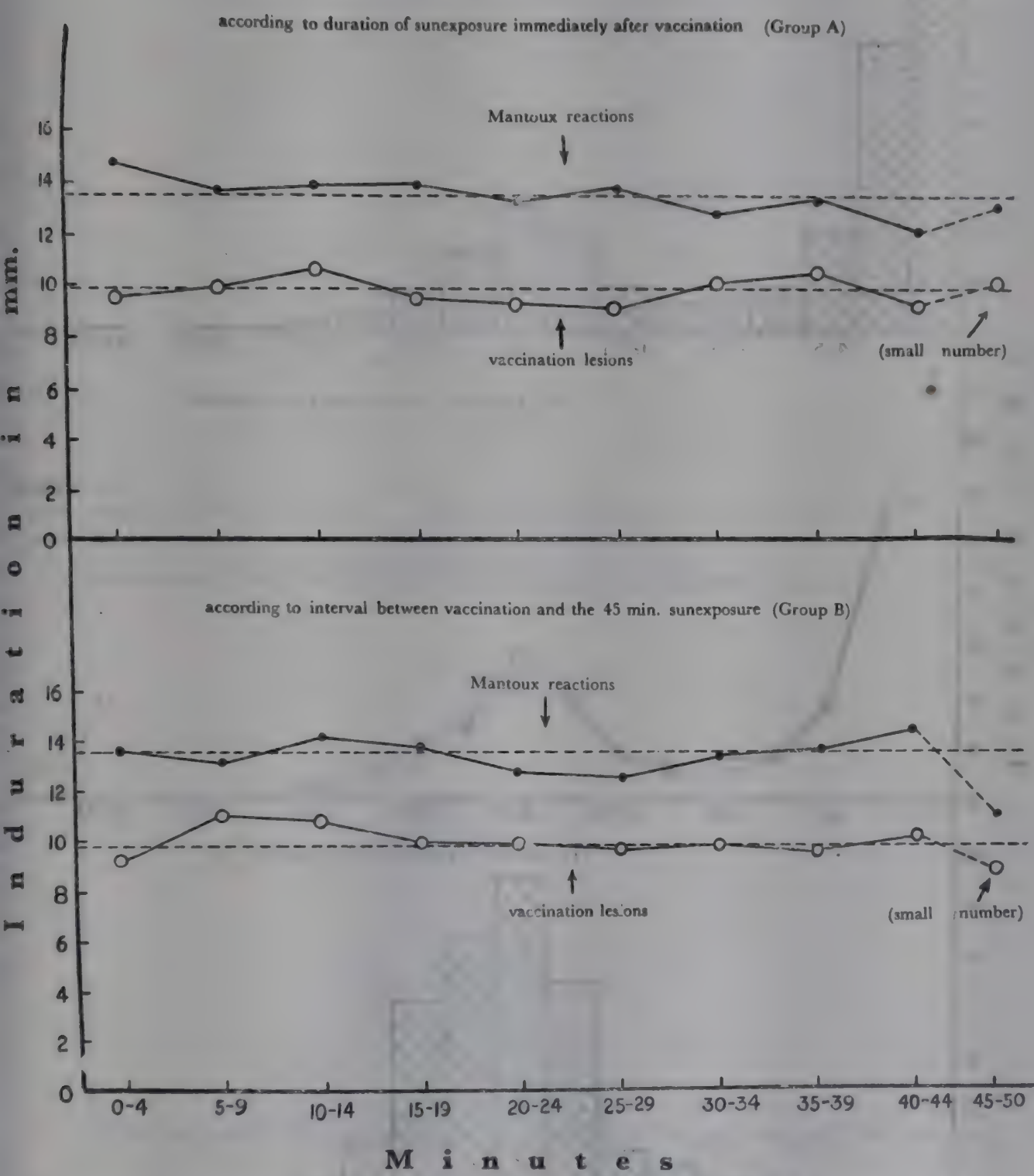


FIG. 2.

Mean size of induration of Mantoux 5 TU reactions and of vaccination lesions
5-6 weeks after vaccination



----- Mean size of induration of Mantoux 5 TU reactions and of vaccination lesions of group C, not exposed to sunlight.

FIG. 3.

Reactions by size of induration to Mantoux 5 TU test.

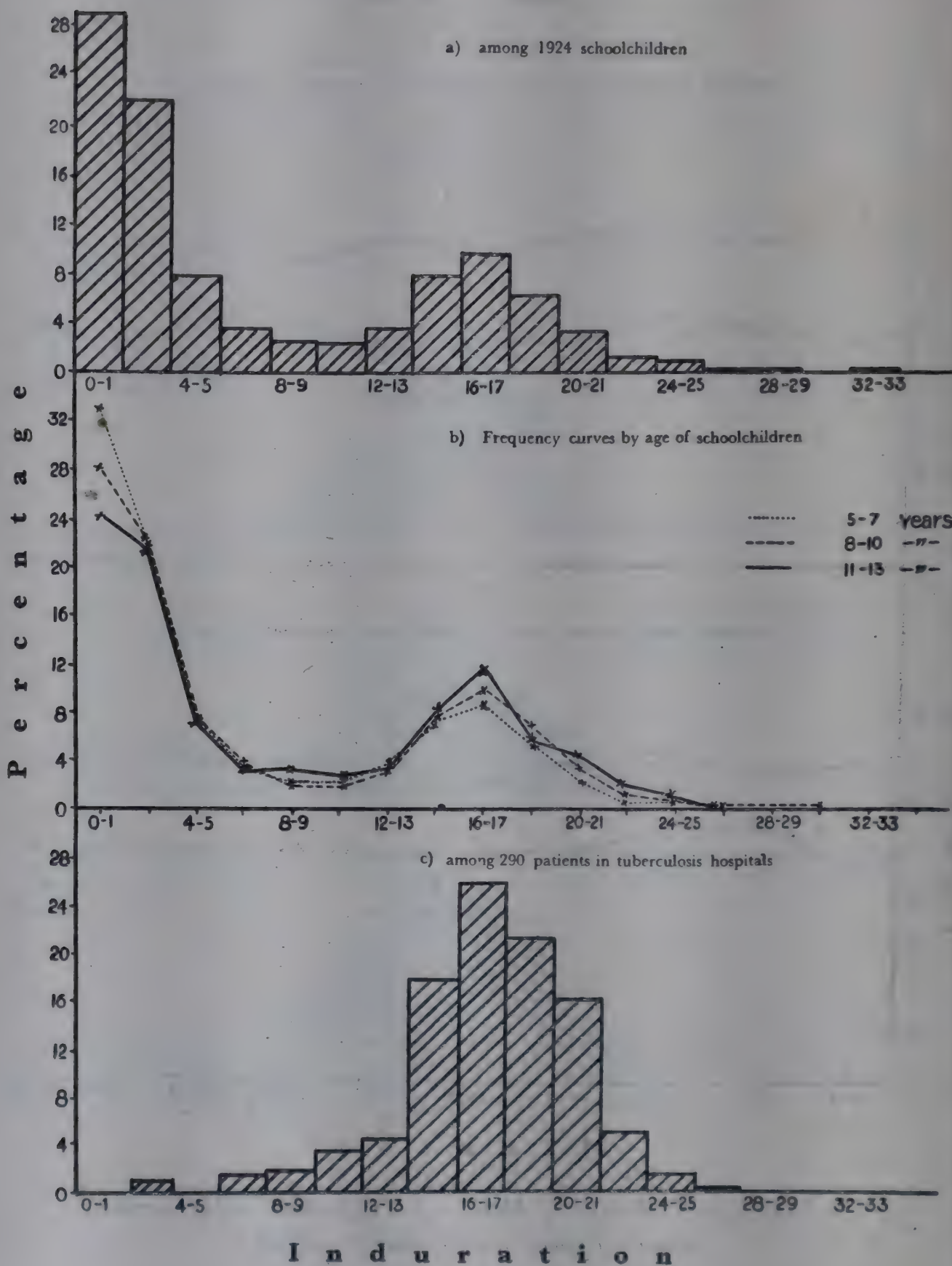
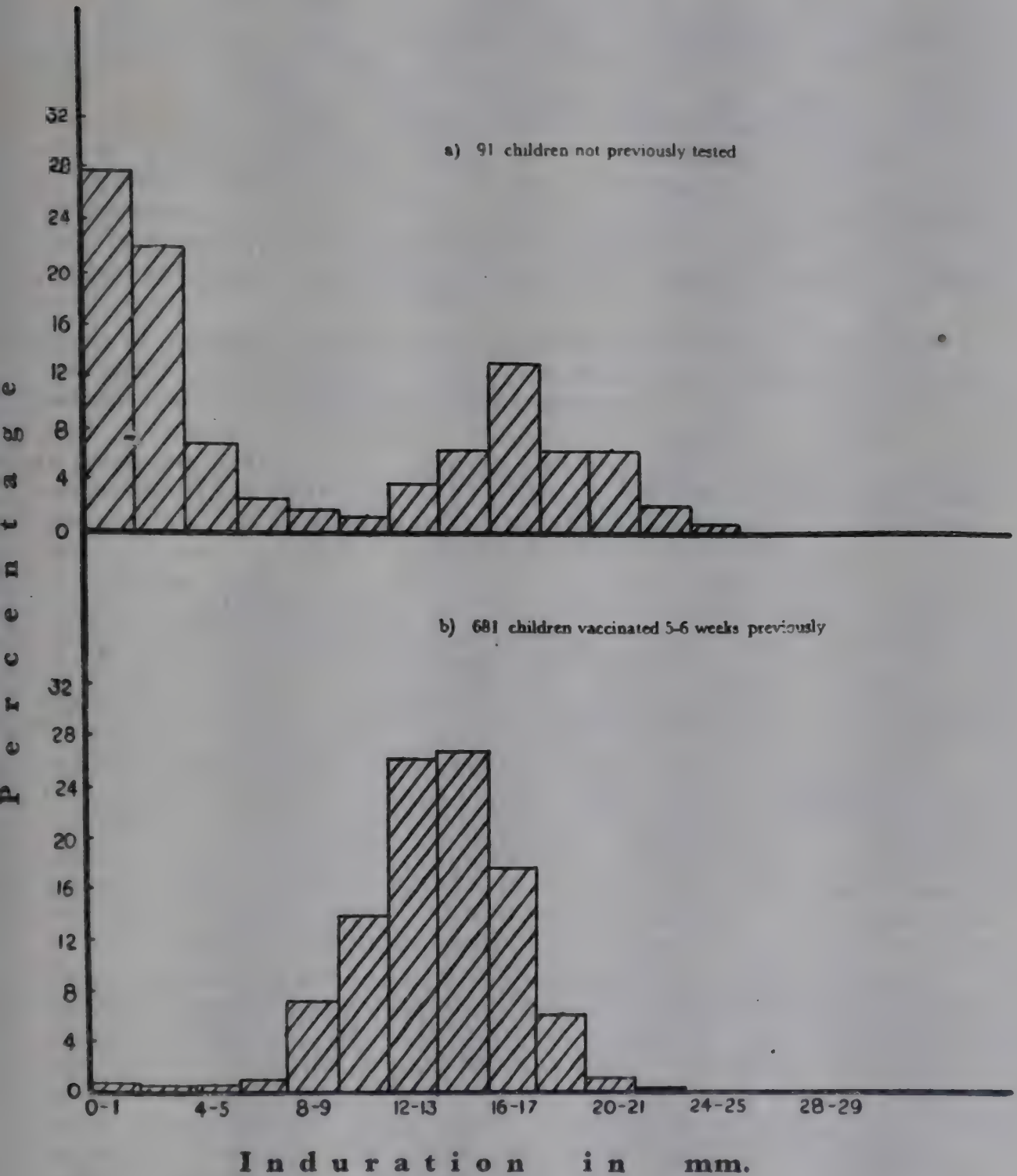


FIG. 4.

Comparison of distributions by size of induration of Mantoux 5 TU reactions in two groups of school children tested at the same time.



32. **Inquiry into the incidence of penicillin resistant strains of *Staphylococcus pyogenes* in Calcutta under Dr. B. P. Tribedi and Dr. J. N. Sarkar at the Medical College, Calcutta.**

SYNOPSIS.

An attempt was made to find out the incidence of penicillin-resistant strains in this part of the country. A number of penicillin-resistant strains were tested against four other antibiotics, i.e. streptomycin, aureomycin, chloromphenicol and terramycin, to find out if the latter antibiotics are of any value in combating the infection caused by the penicillin-resistant *staphylococcus*. Out of 192 *Staphylococcus pyogenes* strains tested, 42 or 22 per cent were resistant to penicillin. Out of 40 penicillin-resistant strains tested against other antibiotics, one (2.5 per cent) was resistant to chloramphenicol, five (12.5 per cent) were resistant to streptomycin and none were found resistant to aureomycin and terramycin. Out of 40 coagulase negative *Staphylococcus albus* tested against penicillin, 28 or 70 per cent were found to be penicillin resistant.

Few factors limit the usefulness of an established antibiotic so much as the development of resistance to it by bacteria originally sensitive. For penicillin this has happened with *Staphylococcus pyogenes*. Barber (1947) working in Hammersmith hospital reported that whereas in 1946 incidence of resistant strains were 14.1 per cent the proportion increased to 38 per cent in 1947 and 59 per cent in 1948. In America, Romanonsky (1949) reported that the incidence of resistant strains of *Staphylococcus aureus*, *Staphylococcus albus* and non-hæmolytic streptococci were 58 per cent, 21 per cent and 55 percent, respectively. Rountree working in Australia reported that in his series 64.7 per cent of *Staphylococcus pyogenes* were resistant to penicillin and that there was an increase of 11 per cent in the incidence of resistant strains from 1949 to 1951. On the other hand, Brinstingle *et al.* (1952) working in St. Bartholomew's hospital have found only 16 per cent of his 200 *Staphylococcus pyogenes* to be penicillin resistant.

In the present work, *staphylococci* were isolated from various pathological material—pus, urine, C.S.F., throat-swab, etc., from in-patients and out-patients of the hospital. No attempt was made to discriminate between patients who had received antibiotics and those who had not. Primary cultures were made on horse-blood agar plates from which colonies were transferred to nutrient agar and broth. Plasma coagulase tests were performed as a routine using rabbit's plasma diluted one in ten.

Sensitivity tests to the antibiotics were made by the streak method on nutrient agar plates in the centre of which heated stainless-steel cylinders were vertically sunk. Cylinders were filled in with antibiotics in proper dilutions and streaks were made from 24-hour broth culture of the test organisms, starting from the edge of the cylinder to the periphery of the plate, using uniform small inoculum in each case. The inoculated plates were immediately put in refrigerator for one hour before they were incubated at 37°C. to allow diffusion of the drugs in the media. Readings were taken after 24 hours. Six strains were tested in each plate, including one of sensitive Oxford strain of *staphylococcus*. The strains were recorded as resistant if growth took place up to the edge of the cylinder, sensitive if

there was no growth within at least 6 mm. from the cylinder. With strains found resistant, tests were repeated using three resistant strains alternating with three streaks of Oxford strain to avoid the uncertainty of diffusion of the drug. Strength of the antibiotics solution used in the actual test were—penicillin 10 units/c.c., streptomycin, chloromycetin, terramycin and aureomycin in the strength of 1 mg./c.c. Solutions were always freshly made, several strains being tested on the same day.

RESULTS.

1. Out of 192 coagulase-positive *Staphylococcus pyogenes* tested against penicillin, 42 (or 22 per cent) were found penicillin resistant. With three results were doubtful.

Thirty resistant strains were re-tested using 400 units/c.c. of penicillin solution in the cylinders (in place of usual 10 units/c.c. solution) and they were still found resistant.

2. Out of 40 penicillin-resistant strains tested against other antibiotics, one (2.5 per cent) was resistant to chloramphenicol and five (12.5 per cent) were resistant to streptomycin. None was found resistant to aureomycin and terramycin.

3. Out of 40 coagulase negative *Staphylococcus albus* incidentally obtained from pathological material) tested against penicillin, 28 (70 per cent) were found to be penicillin resistant.

33. Inquiry on the serological classification of *C. diphtheriae* in Calcutta under Dr. B. P. Tribedi and Dr. D. Barua at the Medical College, Calcutta.

In a previous work Tribedi, Sarkar and Barua (1952) have shown that *C. diphtheriae mitis* is the prevalent organism in Calcutta but in its pathogenic rôle it differed from the *C. diphtheriae mitis* that is found in Western countries being much more virulent. Das and Ghosal (1950) reported the same observation on clinical evidences. Hewitt (1947) and Ferris (1950) in England and Australia, respectively, have done a thorough survey of *C. diphtheriae* on serological basis under the well-established groups, namely gravis, intermedius and mitis. Howitt (1948) suggested that good correlation between serological types and virulence exists. So attempt has been made to explain the difference in pathogenic behaviour of the local strains on serological basis.

The work consists of :—

1. *Isolation and group determination.*—One hundred and ninety-eight strains of *C. diphtheriae* were isolated from the throat and nasal-swabs received from the Medical College Hospitals, Calcutta. Primary cultures were done in Loeffler's slopes and tellurite plate. From the Loeffler's slope morphology was studied and biochemical property tested from the isolated colony from tellurite. Hæmolysis test was done when required. Group was determined by their colony character in tellurite plate and biochemical property. All the strains are found to be *C. diphtheriae mitis*. Eight strains of this series are saccharose-fermenting *C. diphtheriae mitis*.

2. *Raising of high-titre sera.*—Twenty-four-hour old culture of *C. diphtheria* in Loeffler's slant was suspended in 1.0 per cent formal saline and

kept at room temperature for one hour. Then it was centrifugalized and re-suspended in 0.1 per cent formal saline to an opacity of approximately $1,000 \times 10^6$ organisms per c.c. This antigen was tested for sterility and then injected into ear vein of rabbit twice weekly in doses of 0.25 c.c., 0.5 c.c., 1.0 c.c., 2.0 c.c., and 3.0 c.c. One week after the last injection blood was collected from heart and serum separated. Sera with titres of 1 : 250 to 1 : 1,250 were regularly obtained. It was mixed with glycerine in equal proportion and stored at 4°C.

Sera raised did not show any cross matching and was considered to be type specific.

3. *Determination of serological types.*—Five per cent NaCl was used to make a thick suspension of the 24-hour-old culture in Loeffler's slants on a slide. Two suspensions were made, one for control test with five per cent saline alone and another for test to which a loopful (2 mm.) of high-titre serum was added. In positive cases definite visible clumps appeared quickly in less than a minute. So far four serological types have been found.

4. Several representative strains of these four types were tested with the type-specific high-titre sera obtained through the courtesy of Dr. A. A. Ferris of Fairfield hospital, Melbourne, Australia.

Only two types of our series could be agglutinated by his sera and are named according to the nomenclatures used by him, namely 'Bennett' and 'Edmonston 7'. The other two types which could not be agglutinated by any of his sera have been named according to our strain number.

Results.—One hundred and ninety-eight strains have so far been isolated and grouped and 196 strains have been serologically typed. Two strains could not be typed because of auto-agglutination. Of the 196 strains, the types found are :—

137 strains (69.9 per cent)	...	Type '71'
23 strains (11.7 per cent)	...	Type '1574'
24 strains (12.2 per cent)	...	Type 'Bennett'
12 strains (6.1 per cent)	...	Type 'Edmonston 7'

This shows the majority of our strains are of serologically different type from those found in Australia.

MATERNITY AND CHILD WELFARE

1. Inquiry into infant mortality in the Madras State under the Director of Public Health, Madras.

In view of the well-established maternal and child-health organization in the Poonamallee Health Unit area, the inquiry was conducted in that area. It is a typical rural area in Sriperumbudur taluk in Chingleput district, situated about 13 miles from Madras. The area comprised 36 revenue villages extending over 35 sq. miles with a population of 61,523 as per 1951 census.

In order to assess the causes of infant deaths, it was considered that it will be useful to study the health conditions of the mother from the eighth month of gestation. However, as expectant mothers whose period of gestation was more or less than eight months at the time of registration, could not be refused skilled aid from the administrative point of view, they were also registered and followed up. Expectant mothers registered in the maternal and child-health centres of the health unit from 15th May 1951 to 14th May, 1952, were observed for the purposes of inquiry, their confinements attended to as far as possible by the health unit staff and the infants born to these mothers during the period 15th August, 1951 to 14 August, 1952, were observed till 14th August, 1953, when all infants completed the first year of life.

There are six maternal and child-health centres, one in each of the six divisions of the Unit. The area was divided into two parts, each having one Women Medical Officer and three Health Visitors. Each Woman Medical Officer was, therefore, in charge of a population of about 30,000 and each Health Visitor of 10,000. The whole maternal and child-health staff was placed under the administrative control of the Health Officer of the Unit.

The Health Visitor during her home visits, was required, as far as possible, to examine every registered expectant mother once a month before confinement and to refer her immediately to the Women Medical Officer, in case she required medical examination and treatment. The Woman Medical Officer was required to examine each mother clinically once at least during her pregnancy and record her findings in the prescribed schedule. An expectant mother could therefore ordinarily be examined at least four times during pregnancy by the Woman Medical Officer or Health Visitor or the midwife. However, about 25 per cent of expectant mothers could not be examined by the Woman Medical Officer owing to the fact that most of them were registered nearly at full term and the area under each Woman Medical Officer was unwieldy. In these cases, they were visited by the other members of the maternal and child-health staff.

All particulars regarding infant at birth were observed and recorded soon after delivery in the schedule. The infant was generally attended to daily for a period of ten days after birth by the midwives. The Health Visitors were required to visit the infant twice and the Woman Medical Officer once at least during the neo-natal period and thereafter five times by the Health Visitor and once by the Woman Medical Officer. In case of

infant deaths, special visits were paid by the Health Officer and the Woman Medical Officer as early as possible after the event.

A schedule to record the observations of the mother, the infant, their environment, economic status, history of previous pregnancies, conditions of mother during pregnancy and confinement, conditions of infants at birth and during first year of life, was drawn up and printed copies distributed to the maternal and child-health staff. Each schedule will give the history of mother and infant—the mother's record from the date of registration to ten days after confinement, and the infant's record from the date of birth to the date of completion of one year or date of death, whichever is earlier.

The number of expectant mothers registered during the period 15th May, 1951 to 14th May, 1952, was 3,136. Of these, 518 left the area before confinement, and 234 had not confined by 14th August, 1952, with the result, the infants born to these mothers could not be followed up for one year by 14th August, 1953—the date of termination of the inquiry. The mothers who left the area were not a deliberately chosen group, and as such, no bias is introduced by the omission of this group. Out of the remaining 2,384 expectant mothers, the number confined by the Health Unit midwives alone was 1,993 and the rest by barber midwives and hospitals. Of these, 1,503 mothers confined during the period 15th August, 1951 to 14th August, 1952. The number of infants born during this period was 1,511, there being eight cases of twins. Among these, there were 1,460 live births and 51 still births. These 1,460 infants formed the cohort for observation.

Some of the infants under observation left the area before they completed one year of life. In these cases, attempt was made to obtain information as to whether they were alive or dead in the villages or towns to which they have gone. Complete information is not yet available. The number of infant deaths given in this report is, therefore, provisional.

There were 157 deaths among the 1,460 infants under observation. The distribution of infant deaths according to the age groups under one year is shown in Table I :—

TABLE I.
Infant deaths as per age groups.

Age group	Number of infant deaths
Under 24 hours	13
One day and under seven days	20
Seven days and under 30 days	26
Total under one month	59
One month and under three months	36
Three months and under six months	30
Six months and under nine months	14
Nine months and under 12 months	18
Total under one year	157

In view of the fact that complete information regarding the infants that left the area is not yet available, it is not possible to give the infant-mortality rate accurately. A lower limit for infant-mortality rate can however, be set on the assumption that all the infants that left the place are alive at the end of the first year of their lives. It will not be less than 107.5 per 1,000 live births. It is, however, possible to calculate a more accurate infantile-mortality rate by applying the principle of life table, if particulars regarding the number of infants followed up and dropped out of observation at the end of each month of life, are available. These particulars are being compiled. The neo-natal mortality forms 36 per cent of the total infant deaths and represents a rate of 40.4 per cent per 1,000 live births. The rate compares favourably with the rate of 52.7 for the State in 1952.

CAUSE OF INFANT DEATHS.

The causes of infant deaths were determined as pointed out above by inquiry soon after the occurrence of death and on examination of records of previous and present illness. Generally, inquiry was made within seven days and in several cases on the same day of death. In a few instances, the inquiry was conducted later than a week. It may again be repeated that no post-mortem examination was made, and that a more intensive inquiry enabling each infant to be observed every day during the first year could not be carried owing to the limitations set by funds available. An abstract of the causes of infant deaths is tabulated below :—

TABLE II.
Showing causes of infant deaths correlated to ages.

Cause of deaths	Age group							Totals
	0-24 Hours	1-7 Days	7-30 Days	1-3 Months	3-6 Months	6-9 Months	9-12 Months	
1. Birth injury	1	2	1	4
2. Prematurity	5	7	5	2	1	20
3. Congenital deformity	1	2	3	1	7
4. Gastro-intestinal infection	...	1	8	8	15	6	12	50
5. Respiratory infection	...	1	5	9	5	1	2	23
6. Pyogenic infection	1	3	2	...	2	8
7. Other infections	1	3	3	...	1	8
8. Asphyxia	1	8	3	2	...	2	...	16
9. Malnutrition	2	6	4	4	1	17
10. Accident
11. Infective hepatitis	1	1
12. Debility	1	1
13. Cause unknown	...	1	1
14. Virus infection	1	...	1
Total	8	22	29	36	30	14	18	157

Several particulars relating to maternal and infant life have been recorded during the inquiry. Particulars which influence the infant life, namely, seasonal conditions, health and physical condition of mother during pregnancy and at confinement, age of mother, order of birth, interval since the preceding birth, type of delivery, economic conditions, are being compiled and examined and will be dealt with in the final report of the inquiry.

2. Inquiry on the estimation of chorionic gonadotropic hormone in toxæmia of pregnancy with a view to find out the prognostic significance under Drs. Subodh Mitra, Swadesh Basu, Lokenath Bhose, R. Dutt Choudhuri and B. Basu at the Cancer Hospital, Calcutta.

Total number of rats dissected till the 4th September, 1953 was 119. Of them 39 rats were used for preparing the standard curve which was essential for the interpretation of the results in International units. The standard preparation we used was 'Antuitrin S' (Parke Davis & Co., Ltd.).

As we used three rats for each case, we could assay altogether ten normal pregnancy and 13 toxæmia of pregnancy cases till now. All the cases were between 38th and 40th weeks of pregnancy.

Some of the rats died during the process of investigation and we had to discard them.

Technical features.—As the methods and material were discussed in the last report we are giving our revised data below. It may be mentioned that Loraine's Prostatic-weight technique was followed all through the investigation.

<i>Data.</i> — Table I (normal pregnancy)	} See pp. 306, 307 and 308
Table II (toxæmia of pregnancy)	
Table III (average)	
Standard curve	

Standard curve was prepared with the standard preparation of 'Antuitrin S' by injecting it in divided and increasing doses and noting the increase in prostatic weight in nine groups of rats.

Comments.—Dr. Loraine's observation was based on 45 cases. Our results on ten normal cases and 13 toxæmia cases do not compare favourably with Dr. Loraine's results.

Our normal range in ten normal cases is between 117 I.U. and 919 I.U. (average—396 I.U.) and in 13 toxæmia cases between 438 I.U. and 11,000 I.U. (average 3,459 I.U.).

In our series of 23 cases, it was not possible to find a line of demarcation between the high normal and the low toxæmia values.

We wrote to Dr. Loraine in Edinburgh about our results and he advised that constancy in the weight of the rat was more important than the constancy in the age of the rats. Since then we are trying to keep the weight of the rats more or less constant irrespective of the age of the rats but at the same time keeping it in mind that the importance of the investigation lies in the immaturity of the rats. Unfortunately the results did not change appreciably.

Another factor seemed significant for this discrepancy. One of us saw in Edinburgh that in Dr. Loraine's series the average body-weight of the immature rats was between 40 g. and 50 g. and sometimes more when they were 20 to 23 days old. For the same age, the average weight in our series was 24 g. Whether this retardation of growth was due to the climate in spite of the balanced diet and air-conditioned-house allotted for them, is not clear. This retardation in the general growth would certainly affect the growth of the prostate even when the rat was treated with a growth stimulus like Chorionic gonadotropin.

The results shown in Tables I, II and III are, however, based on the animals locally bred in our animal house. We have observed, as Dr. Loraine has also observed, that there is often a significant rise in the chorionic gonadotropic content of urine of some of the toxæmia of pregnancy cases.

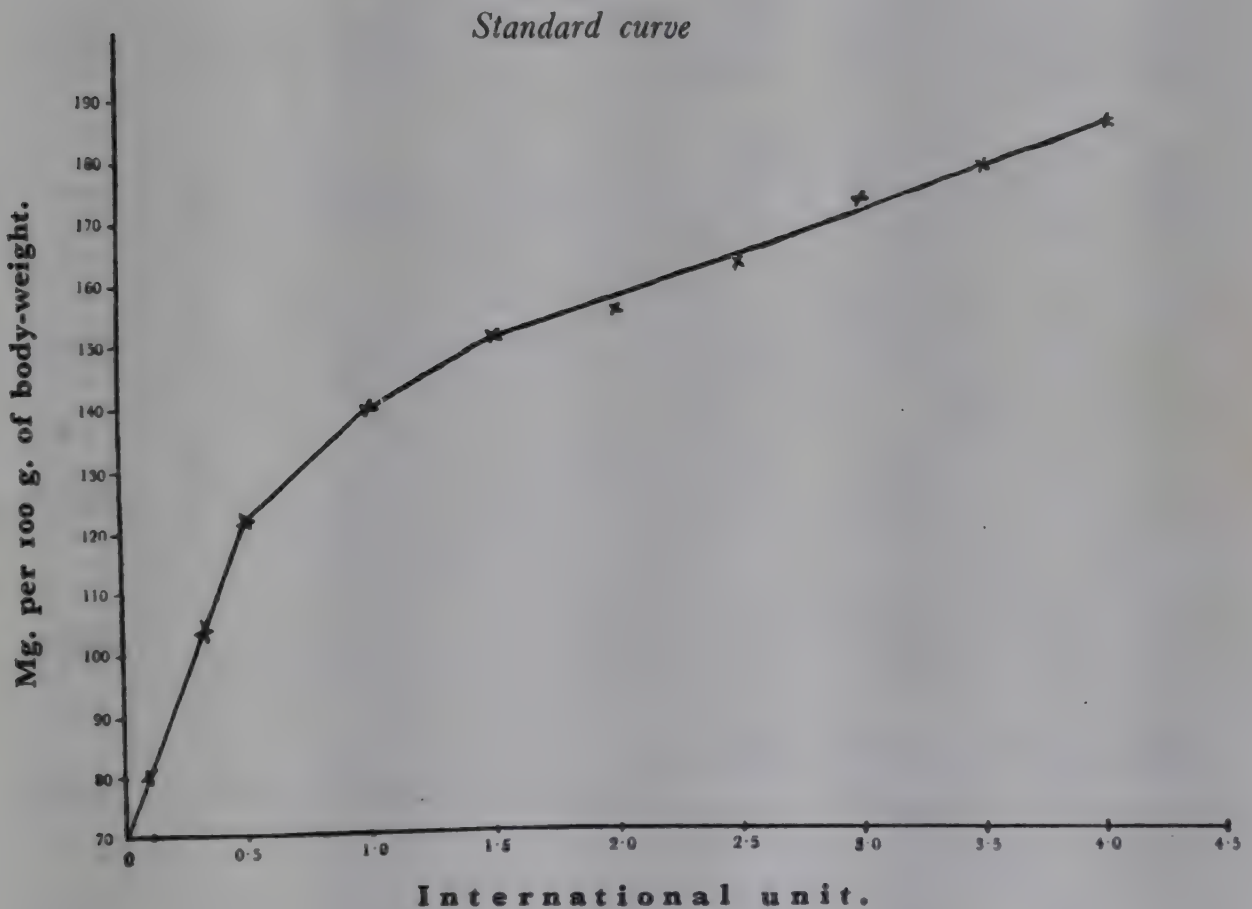


TABLE I.

Detailed data of 30 rats treated with urine of ten cases of normal pregnancy.

Serial number	*B.-wt. in g.	†P.-wt. in mg.	P.-wt./100/g of B.-wt.	Mean P.-wt. per 100 g. of B.-wt.	P.-wt. inter- polated in I.U.	Total quantity of urine in c.c./ 24 hours	24 hours excre- tion of C. G. in urine
1	16.5	11.7	71	66.6	0.02	2,927	117.08
2	20.5	10.2	49.8				
3	24.1	19.0	79				
4	28.0	19.3	69.2	71.4	0.04	3,443	275.4
5	23.5	11.0	81				
6	20	12.8	64				
7	25	22.5	90	82.9	0.12	1,558	373.9
8	30	30.3	101				
9	40	23.0	57.7				
10	23	19.0	87	77.5	0.09	1,727	310.8
11	25	17.6	70.5				
12	25	18.7	75.0				
13	25	17.8	71.3	85.1	0.15	2,700	810.0
14	32	25.6	80.0				
15	33	21.0	64.0				
16	19	15.3	80.7	87.9	0.16	2,872	919.0
17	25	22.7	91.0				
18	29	32.5	112.0				
19	20	18.4	92	80.1	0.1	1,211	242.2
20	30	21	70.3				
21	27	22.4	78				
22	28	18.7	80	76.7	0.05	2,660	266.0
23	25	23.3	75				
24	31	20	75.1				
25	25	20	80	80	0.1	1,058	211.6
26	30	21.0	70				
27	28	25.2	90				
28	21	21	100	83	0.11	2,000	440.0
29	27	21.3	70				
30	35	24.5	70				

*B.-wt. = Body-weight.

†P.-wt. = Prostatic-weight.

TABLE II.

Detailed data of 39 rats treated with urine of 13 cases of toxæmia of pregnancy.

Serial	*B.-wt. in g.	†P.-wt. in mg.	P.-wt./100/g. of B.-wt.	Mean P.-wt. per 100 g. of B.-wt.	P.-wt. inter- polated in I.U.	Total quantity of urine in c.c. /24 hours.	24 hours excre- tion of C.G. in urine.
1	20	22.4	112	112	0.42	595	495.8
2	18	18	100				
3	22	27.2	124				
4	23	28.7	125	108.6	0.38	1,331	1011.5
5	23	27.5	110				
6	21	27.7	90.8				
7	16	20.8	130.6	143.9	1.1	523	1150.6
8	20	30	150				
9	24	36.2	151.1				
10	22.5	25.2	112	108.3	0.36	2,020	1454.4
11	20	20.2	101.3				
12	21.3	23.7	111.6				
13	19.5	31.2	160	154	1.65	1,100	363.0
14	20	30	150				
15	23	34.9	152				
16	27	40	148	148	1.3	2,708	7040.8
17	24	36.2	151.1				
18	20	28.9	144.9				
19	23	23	100	89.2	0.2	2,873	1149
20	28.5	25.5	90				
21	21	16.3	77.6				
22	33	56.2	168	170	2.8	1,738	9732.8
23	24	36.1	151				
24	30.5	58.2	191				
25	32	54.5	170.4	170.3
26	25	45	180.2				
27	21.5	34.4	160.3				
28	38	33.2	87.4	86.4	0.15	3,000	450
29	28	28.8	103				
30	25.5	17.8	70				
31	24	33.6	140	137	0.9	1,500	2700
32	31	37.2	120				
33	33	49.8	151				
34	33	29	126	150	1.4	1,527	4275.6
35	42	73	174				
36	35	52.5	150				
37	27	28	102.1	103.7
38	34	36.7	108				
39	26.4	26	101				

*B.-wt. = Body-weight.

†P.-wt. = Porstratic-weight.

TABLE III.

Mean prostatic weight ratio and the excretion of C.G. per 24 hours in ten normal and thirteen toxæmia of pregnancy cases.

Cases		Number of rats dissected	Average B.-wt. in g.*	†P.-wt./100 g. of B.-wt.	24 hours excretion of C.G. in I.U.
Nature of treatment	No.				
Treated	X	11	21	65·7	X
Treated with normal pregnancy urine ...	10	30	26·3	79·12	396·6
Treated with toxæmia of pregnancy urine ...	13	39	24·76	129·33	3459·09

*B.-wt. = Body-weight.

†P.-wt. = Prostatic-weight.

3. Investigation on the problem of late abortions from four to seven months of pregnancy under Dr. H. V. Tilak at the Topiwala National Medical College, Bombay-8.

During the year, 480 cases were investigated. It was stated at the start of this inquiry that cases of abortions occurring between four and seven months of pregnancy would be investigated. Actually it was found more reliable to take up cases of pregnancy of 16 weeks to 30 weeks. The fortnight from 14th week to 16th week was given up to eliminate most of the cases with a doubtful period of amenorrhoea. Even so, 37 cases were found early abortions as determined by a careful calculation of the date of the last menstrual period and measurement of the fresh foetus when available. Similarly, 15 cases of missed abortions were detected where the foetal measurement showed that the foetus must have ceased growing before 16th week. There were also 31 cases of mistaken diagnosis or with extremely poor record. After excluding all these 83 cases this report is based on 397 cases only.

Every attempt was made to secure the products of conception in each case but in some cases they were not available because the mothers aborted at home or their relatives claimed the products of conception. This did not matter much when the cause of abortion could be established by other evidence, such as syphilis, vesicular mole, criminal abortion, definite trauma, infectious fevers, etc., but the products of conception were not available in 95 cases where no such evidence as to the cause of abortion was found.

Stool reports were obtained in 182 cases only as some mothers left the hospital on the 2nd day of admission. Blood reports for hæmoglobin test were available in 331 cases for the same reason or when the mothers refused

to be pricked. Letters and reminders were sent to their husbands both to come to the out-door for these tests and also for the local examination of the wives and the blood and semen examination of husbands ; but the response was poor. Thus, Kahn test or test was done in the case of mothers in 274 cases and of husbands in 71 cases only. Semen examination was done in 50 cases only.

Control cases.—It was mentioned in the interim report that it was decided to investigate the incidence of anæmia, syphilis, toxæmia and placental and foetal defects in consecutive labour cases with live births to serve as control for the cases of late abortions with a view to see whether the proportion of these factors is significantly large in cases of late abortions. Three hundred and sixty-four cases were so investigated till the end of July 1953. In these cases complete investigation of blood, stools, etc., could not be done in all of them either because many delivery cases were discharged on the 3rd or 4th day or due to the non-co-operative attitude of the patient. Follow-up response was not satisfactory. The percentage result of each factor is, therefore, drawn from such cases only in which the necessary tests could be done. The Tables give the figures for cases of late abortions and labour cases to facilitate comparison.

Defects in placenta and membranes.—The following six types of defects were noticed : (1) infarcts, (2) retroplacental hæmorrhages, (3) clots near the margin, i.e. near the circular sinus of placenta and behind the membranes, (4) thin and/or extensive placenta, (5) a tongue-line projection at one side of the placenta. This portion was usually free from membranes and was connected with blood clots. Types 4 and 5 may be called placenta membranacea and (6) placenta circumvallata.

These types were not usually found singly but in various combinations. Types 1, 2 and 3 are also found in placenta in the control delivery cases and so they are not taken into consideration in this report. Twenty-six cases of types 4, 5 and 6, i.e. placenta membranacea and placenta circumvallata, were found in the present series of late abortions and not a single case of these types in the 364 control delivery cases. This suggests that they may be the cause of late abortions in many of these cases, though in this report they are not classified as an established cause of late abortion. This condition seems to be the end-result of a cause that is still to be determined. Such placenta may be due to faulty decidual reaction or some defect in the implantation of the embryo. Further investigation is in progress.

Defects in the cord.—In 35 cases in this series cord was found with innumerable twists and shrinkage like a thread. This must result in the death of the foetus, though the cause of such twists is not yet understood. Javert and Barton (*Amer. Jour. Obstet. & Gynecol.*, May, 1950) have found the incidence of various cord abnormalities eight times more in spontaneous abortions than in therapeutic and incidental abortions. This condition is also under further investigation.

Semen defect.—According to Mazer and Esrael, if the semen contained 25 per cent or more abnormal sperms the embryo of such union is very likely to die or be abnormal. In the 50 semen examinations done, this defect was found in 19 cases. The high abortion and still-birth rate and other complications associated with this defect are shown in the Tables I and II ;—

TABLE I.

Number of semen defects.	Average age of mother.	1st gravidac.	Abortion and still births.	Premature delivery.	Placental defect.	Cord defect.	Twins
19	21	8	22	3	7	4	1

TABLE II.

Investigation on the problem of late abortions from four to seven months of pregnancy

Cases of late abortions.

Delivery cases as control.

Total investigated upto 30-6-53...397

Total investigated...364

	Number available.	Number of defects.	Percentage.	Number available.	Number of defects.	Percentage.
1. Placenta membranacea and P. circumvallata ...	191	26	13·61	362	Nil	Nil
2. Cord defects ...	173	35	20·23	362	Nil	Nil
3. Anæmia Hb 50 per cent or less	331	75	22·7	339	63	18
4. Bowel infestation :						
(a) Roundworm ...	182	60	33	266	82	38
(b) Ankylostoma ...	182	11	6	266	14	5
(c) Amœbic dysentery ...	182	5	2·75	266	4	1·5
5. High blood pressure, more than 120 mm. systolic ...	397	10	2·5	364	26	7·9
6. Syphilis in mother ...	274	22	8	274	8	3
7. Toxæmia ...	397	9	2·26	364	10	2·7
8. Twins ...	173	10	5·7	364	2	·5
9. Placenta previa and abruptio placentæ ...	397	12	3	364	5	1·4

Twins are known to be a cause of abortion in many cases. Ten cases of twins were found in 173 cases of embryos that were available for inspection giving an incidence of 5·7 per cent. In 364 delivery cases, only two cases of twins were found.

In this series cause of late abortion was considered to be established, if evidence of (i) therapeutic or criminal abortion, (ii) physical or mental trauma, (iii) syphilis, (iv) vesicular mole, (v) monsters, (vi) twins, (vii) toxæmia, (viii) abruptio placentæ or placenta previa, (ix) defects of the cord, (x) defects of semen, (xi) infectious fevers, and (xii) iso-immunization was found. Such evidence was found in 161 cases of late abortions, i.e. 40·25 per cent of the total. If the examination of products of conception, of blood for syphilis and of semen were possible in every case, cause of late abortion would have been established in some more cases. The number of criminal abortions is also indefinite as it is very difficult to get the correct history in every such case.

Iso-immunization.—Dr. L. D. Sanghvi, Ph.D., and Shri H. M. Bhatia, both working now in the Indian Cancer Research Centre, Bombay, have examined upto now 154 cases for ABO, MN and Rh blood groups of mothers and the aborted foetuses. Their results are as follows :—

Iso-immunization due to Rh	...	Nil
„ „ „ anti-A	...	1
„ „ „ anti-B	...	2

Certain devitalizing and complicating factors.—It was thought that a combination of certain factors might cause abortion. Thus, the following factors may increase the possibility of late abortions if three or more are found present in the same case :—

(1) Young primipara under 16, (2) multipara with eight or more deliveries, (3) previous late abortions or still births, (4) vomiting for two months or more, (5) anæmia with Hb 50 per cent or less, (6) prolonged diarrhoea, (7) fever of more than five days duration, (8) hypertention, (9) lung disease, (10) heart disease, (11) placental defects : (a) infarcts, (b) blood clots, (c) membranacea and circumvallata, (12) local conditions : (a) deep lacerations, (b) prolapse, (c) previous operation on womb, and (13) history of menstrual abnormality.

Among the 236 cases in which no definite cause was detected, 24 cases with such combinations were found. This high incidence is a pointer and it is to be seen whether prevention of such cases will lower the incidence of late abortions. These factors will be investigated in labour cases with live babies and their incidence will be compared with those found in late abortions.

4. Inquiry into the incidence and causes of still births and neo-natal deaths in Hyderabad under Dr. (Smt.) P. M. Naidu at the Osmania Medical College, Hyderabad-Deccan.

Since the commencement of work, 82 cases of still births and 77 cases of neo-natal deaths were studied ; but of these autopsies were performed

on 26 cases (still births 18, neo-natal deaths eight). The causes of still births and neo-natal deaths as observed here are shown in Tables I and II.

TABLE I.

Classification of causes of still births and neo-natal deaths studied at the Osmania Hospital, Hyderabad-Deccan.

Cause of still birth or neo-natal death	STILL BIRTHS		NEO-NATAL DEATHS		Remarks
	Total cases studied	Cases in which autopsy was performed	Total cases studied	Cases in which autopsy was performed	
Gross errors of development	7	3	7	2	
Asphyxia ...	30	7	4	1	
Birth injuries, including intracranial hæmorrhage	2	...	6	1	
Toxæmia and nephritis	13	2	
Other constitutional diseases of mother ...	4	4	
Infections	
Syphilis ...	14	2	
Erythroblastosis	
Prematurity	58	3	
Miscellaneous ...	1	...	1	1	
Undetermined ...	11	...	1	...	
Totals ...	82	18	77	8	

TABLE II.

Causes of still-births and neo-natal deaths in different hospitals.

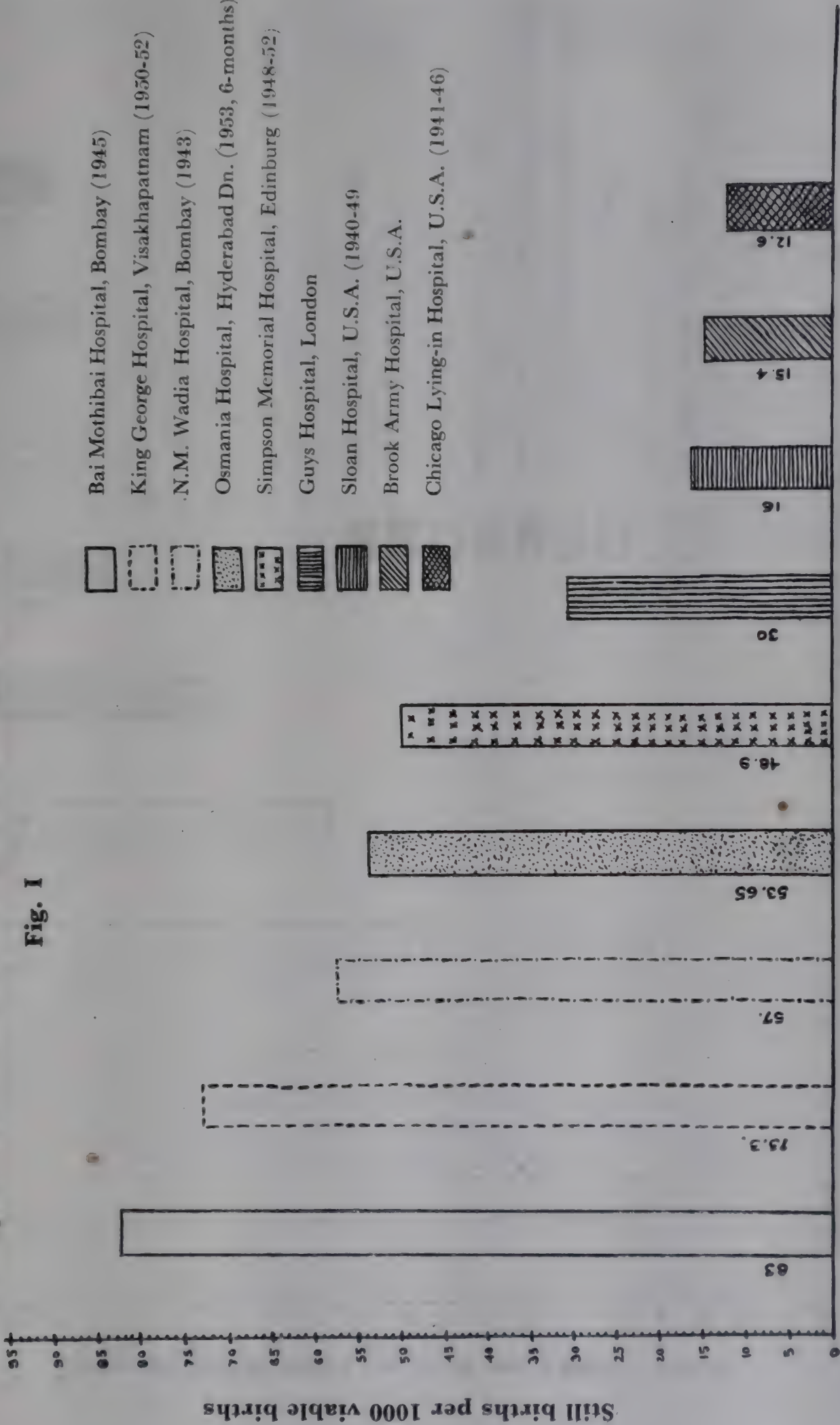
Causes of still births and neo-natal deaths	GUY'S HOSPITAL, LONDON (1)		SIMPSON MEMORIAL HOSPITAL, EDIN. (2)		CHICAGO LYING-IN HOSPITAL, U.S.A. (3)		KING GEORGE HOSPITAL, VISAKHAPATNAM (4)		OSMANIA HOSPITAL, HYDERABAD-DN. (5)	
	Still births per- centage of 337 cases	Neo-natal deaths percentage of 140 cases	Still births percentage of 422 cases	Neo-natal deaths percentage of 338 cases	Still births percentage of 221 cases 1941-46	Neo-natal deaths percentage of 219 cases 1931-46	Still births percentage of 106 cases	Neo-natal deaths percentage of 56 cases	Still births percentage of 82 cases	Neo-natal deaths percentage of 77 cases
1	2	3	4	5	6	7	8	9	10	11
Gross errors of deve- lopment ...	6.5	7	20.85	1.59	10	2.9	8.5	8.92	8.5	9.1
Asphyxia ...	36.8	10	50.94	26.9	38	10	42.37	17.7	36.5	5.19
Birth injuries ...	17.4	8	33.65	39.8	1.8	1.2	5.66	10.77	2.43	7.7
Toxæmia and nephritis ...	10.98	7.18	...	17	3.5	15.8	...
Other constitutional diseases of mother ...	0.89	9	...	4.9	...
Infection	11.4	4.5	40.5	1.3	8.4	3.77	42.8
Syphilis ...	0.59	0.9	...	3.77	1.7	17.0	...
Erythroblastosis ...	1.21	8.1	1.5	0.94
Prematurity	54.0	75.3
Miscellaneous	10	0.9	1.5	2.82	14.2	1.2	1.3
Undetermined ...	26.1	...	14.93	3.5	31.2	...	15.0	...	13.4	1.3

From Table I it will be apparent that asphyxia was the cause of still births in 30 out of 82 cases (36·5 per cent), syphilis in 14 (17 per cent) and toxæmias in 13 (15·8 per cent). The cause of asphyxia in the majority of cases was due to mechanical impediment to labour, such as breech presentation, poor uterine contraction, deformed pelvis, malposition and presentation of the fœtus. The diagnosis of syphilis is based upon the history of previous pregnancies and a positive Wasserman reaction. Wherever possible, this was confirmed by autopsy and histopathological examination of placenta and viscera of the affected fœtus. In 11 (13·4 per cent) the causation of still birth was undetermined.

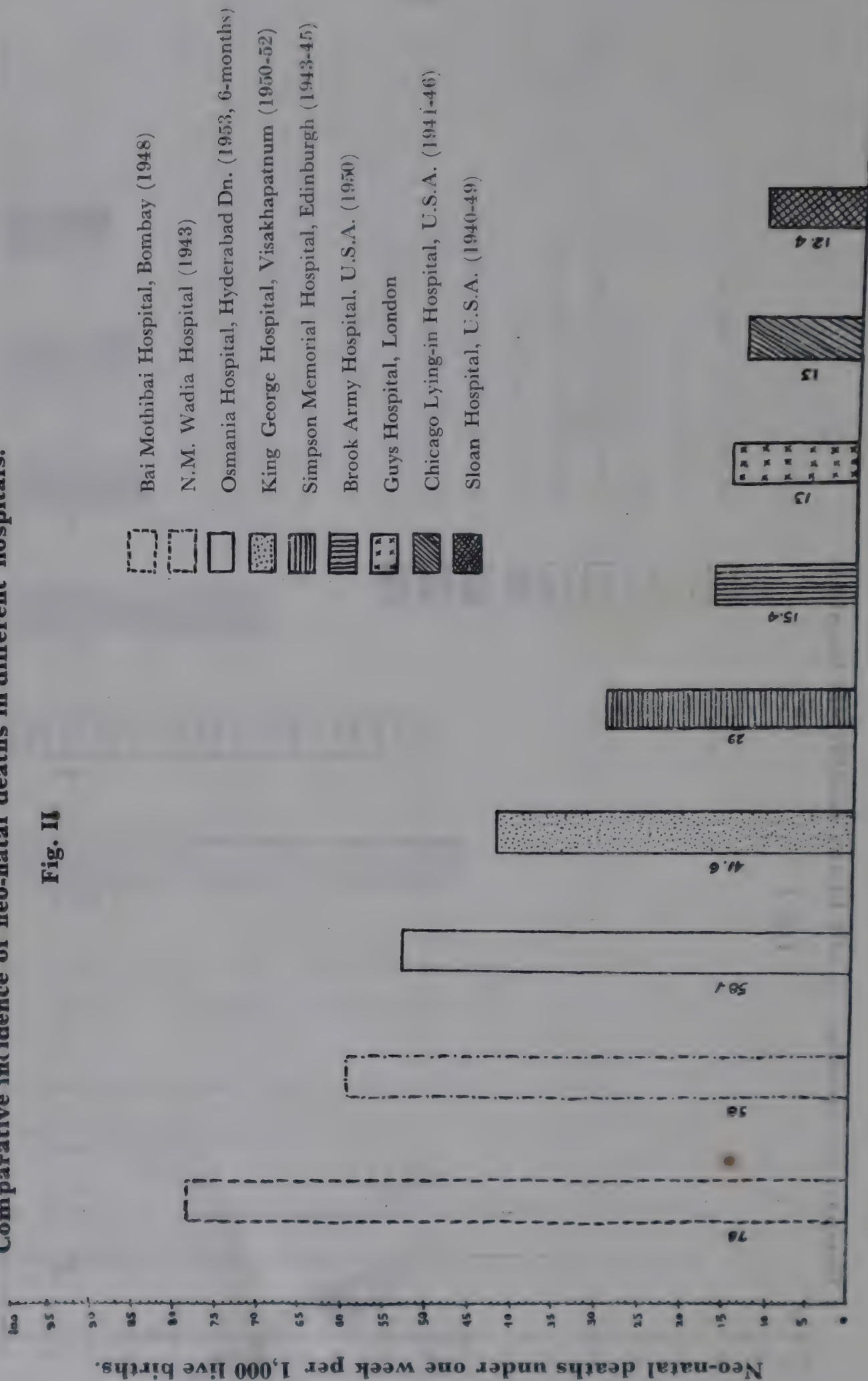
Among the neo-natal deaths a high percentage of cases has been classified, for the present, under 'prematurity' according to the usually accepted definition of the term. Ætiologically, a considerable number of these cases could be attributed to such constitutional diseases of the mother as severe anæmia, toxæmia, syphilis and malnutrition. In one case the causation of death was undetermined.

Comparative incidence of still births in different hospitals

Fig. I



Comparative incidence of neo-natal deaths in different hospitals.



5. Inquiry on the structural and functional adaptation of the kidneys in pregnancy under Dr. Chunilal Mukherjee at the Medical College, Calcutta.

Preliminary reconnoitering experiments were done on ten cases and standards checked in order to determine the reliability of the results obtained.

Biochemical estimations and investigation formed the first part of the study. So far 12 cases of normal pregnancy and three normal non-pregnant females of reproductive age have been investigated. The investigation, at the present stage, consists of determination of renal clearance rates for urea, uric acid, endogenous creatinine, chloride, phosphate, sodium, potassium and calcium. The methods employed for this determination were those of E. J. King as described in 'Micro-analysis in Medical Biochemistry'. The standard formula, viz. $U/B \times V$, was employed for the determination of clearance. Twenty-four hours' output of urine was obtained in each case and the minute volume calculated therefrom. Height, weight and surface area of each subject has been calculated. All patients with evidence of organic diseases have been eliminated. The number of cases done so far is yet small, the range of values obtained are given below :—

	Normal pregnancy c.c./minute	Control c.c. /minute
Endogenous creatinine ...	89-132	98-120
Urea ...	27.8-46	42-56
Uric acid ...	50-64	64-78
Chloride ...	0.5-1.1	0.7-1.4
Phosphate ...	11.2-15.7	11.0-15.0
Sodium ...	1.1-1.3	1.0-1.1
Potassium ...	11.2-14	9.1-12.6
Calcium ...	0.4-0.7	0.7-0.9

These results have not been corrected for surface area, height or weight owing to the smallness of the series. The individual values for the ingredients mentioned above in all cases in both urine and serum were within normal limits.

6. Research on radiological electro-cardiographic changes in normal pregnancy under Dr. (Kumari) S. Padmavati at the Lady Hardinge Medical College, New Delhi.

Twenty-five patients with normal hearts and three with organic heart disease are being studied at the present time. It was difficult to get a cardiac patient in the early months of pregnancy, hence the small number in this series. The cases are seen every month. They were first studied in most cases at eight weeks pregnancy and in a few cases at 12 to 14 weeks. One patient has been followed up as far as the 34th week. The others are all much less advanced in pregnancy.

ELECTRO-CARDIOGRAPHIC FINDINGS.

The following features were noted in the electro-cardiogram :—

1. *Standard leads*.—There was no significant alteration from the normal in the rate, rhythm, duration of PR, QRS or QT.

2. *Axis deviation*.—Twenty-one patients had a normal electrical axis. One patient showed slight right-axis deviation. The unipolar limb leads in this case showed a vertical heart. These cases showed left-axis deviation. One had a semi-horizontal position of the heart as judged by the unipolar limb and chest leads. In the other two the left-axis deviation was not accounted for. There was no change in the axis with the advance of pregnancy.

3. *T-wave changes*.—Inverted T waves in lead iii were seen in three cases, flat T waves in the same lead in 13 cases, and bi-phasic T in two cases. A deep Q in lead iii was present in only one case, and this was observed at her very first visit during the third month. Q waves of significance in lead iii have not been noticed in any of the other cases. This appears to contradict the findings of previous workers, but perhaps it is too early to say this.

4. *Unipolar limb leads*.—In 14 cases the heart occupied an intermediate position. There was no change with increase in size of the uterus, except in one case in which at 24 weeks the heart changed to a semi-horizontal position.

In four cases, the heart was vertical with clock-wise rotation. There was no change with advancing pregnancy, at least as observed so far.

In six cases, the heart was semi-vertical in position to begin with. Four of these showed a gradual shift in position. In three of them the heart assumed an intermediate position at periods varying from 18 to 28 weeks. In one case the position became vertical at 15 weeks.

5. *Chest leads*.—No abnormality has been observed so far in the chest leads apart from a shift in the transition zone with a change in position of the heart.

Thus, in one patient with a vertical heart, the transition zone changed from V3 to V2 at 12 weeks pregnancy, when the heart assumed an intermediate position. In another patient with a semi-vertical heart which changed to vertical at 16 weeks, TV3 which was formerly inverted became upright.

CONCLUSIONS.

It would appear that the changes in the ECG during pregnancy are mostly in the nature of a shift in position. The significance of this shift and its relation to the height of the uterus, it is difficult to assess at present. The axis deviations are of interest and worthy of finding an explanation for. The exact relation between the position of the heart and the transition zone also needs to be worked out.

7. Inquiry into the incidence of intra-natal and early rickets and their ætiology with special reference to mother's milk and her diet under Dr. B. D. Patel, Dr. S. D. Soman, Dr. B. D. Patwardhan and Shri N. C. Datta at the Grant Medical College, Bombay.

The following procedure was adopted in carrying out the investigation :—

Cases were selected at random from pregnant women in the seventh month of gestation, from the Ante-natal Department of Bai Mothibai Hospital. Records were kept of previous pregnancies, dietetic history with special reference to their intake of calcium and phosphorus and of their general state of health.

Blood was collected from those pregnant women first at the seventh month's gestation period and then soon after delivery. The cord blood of the newborn was also collected immediately after delivery. The determination of calcium, phosphorus and phosphatase activity was carried out in all the samples of blood.

By a process of close questioning, attempt has been made to ascertain the nature and the amount of diet consumed and from this the intake of calcium and phosphorus has been calculated. It is admitted that this procedure does not give accurate results, yet it may serve to give a fairly rough estimate of the intake of the elements. It is, however, proposed to analyse random samples of their diet for the intake of calcium and phosphorus.

A clinical examination was done of the new-born for the evidence or otherwise of rickets, special attention being paid for the presence of craniotabes, prominent epiphysis, thickened costochondral junction and any abnormal curvature of the bones of the extremities.

On the second day of the neo-natal period the wrist and the skull were x-rayed for an evidence of rachitic changes.

Subsequently, these new borns were kept under observation for a period of three months and monthly checks up were done for the evidence of rickety process. At monthly intervals, skiagrams of the wrist and skull were taken and the findings recorded. The x-rays are submitted to experienced radiologist for the assessment of the rachitic changes. For control studies similar investigations were carried out on full-term healthy babies with birth-weight above six pounds.

Samples of mother's milk are collected on the fourth day and thereafter at monthly intervals for the estimation of calcium and phosphorus content.

Those mother's who had their calcium, phosphorus and phosphatase content below normal level had their skiagram of the pelvic bones taken for the presence or otherwise of osteomalacia.

RESULTS AND DISCUSSION.

This preliminary report includes the results of investigation carried on 60 expectant mothers. This series consisted of 12 primipara, 41 from second to sixth para and seven from seventh para and above. All these

cases belong to poorer classes and as such displayed clinical evidence of anæmia, general malnutrition and symptoms of avitaminosis, particularly of vitamins A and B complex. Most of them showed history of intestinal disorders of the nature of either constipation or frequency of stools.

Calcium and phosphorus intake.—The calcium and phosphorus intake as calculated from food intake was 0.338 g. and 0.785 g. per day, respectively. These values appear to be far below the level recommended for pregnant women.

Calcium, phosphorus and phosphatase content of the blood of pregnant women at seventh month of gestation and after delivery.—Of the 60 cases followed from seventh month of gestation, 16 delivered. It was possible to investigate 13 of these 16 cases.

Blood calcium.—The calcium content of blood serum at seventh month of gestation varied from 7.2 mg. to 11.9 mg. per 100 c.c. Twenty-five of the 60 cases had calcium below 9 mg. per 100 c.c., of which five had below 8 mg. per 100 c.c. Values between 9 mg. and 10 mg. per 100 c.c. were found in 25; the remaining ten cases had values above 10 mg.

The calcium content of the 13 mothers who delivered was slightly on a higher range as compared with those at seventh month of gestation and ranged between 8.3 mg. and 12.4 mg. per 100 c.c. In five of these cases the serum calcium varied between 8 mg. and 10 mg.; in five others it varied between 10 mg. and 11 mg. per 100 c.c. Two cases had calcium below 9 mg. and two above 11 mg. per 100 c.c.

Blood phosphorus.—The blood phosphorus ranged between 2.1 mg. and 5.5 mg. per 100 c.c. at seventh month gestation. In all except five cases, the blood phosphorus was within normal limits. There was little change in serum phosphorus at delivery and remained normal in all except one.

Serum phosphatase.—The serum phosphatase in mothers at the seventh month of gestation was within normal limits, while at full term there was shift towards a higher level of normal. Values above normal were observed in six mothers at full term, the highest value recorded being 19.2 Bodansky units.

Calcium, phosphorus and phosphatase contents of cord blood of 13 new born babies.—The average calcium content of cord blood was found to be 9.98 mg. with a range varying between 8.6 mg. and 11 mg. per 100 c.c. The phosphorus ranged between 3.4 mg. and 7.6 mg. with an average of 5.38 mg. per 100 c.c. All the values of calcium and phosphorus appear to be within normal limits except in one case where they were below normal.

The alkaline phosphatase values of all the new borns except in one was within normal limits. The highest value of 18.5 Bodansky unit was found in the case of a new born whose mother showed also the highest value among those who delivered. The calcium and phosphorus contents of the cord blood were 9.5 mg. and 5.6 mg. per 100 c.c., respectively, and are normal. The phosphatase value of this new born, however, fell to 5.7 units by the sixth day. The skiagram of this infant failed to show any rachitic process at the wrist joint or in the skull bones.

The calcium and phosphorus values of the new borns tended to be slightly higher than those of the mothers, thus being in conformity with the observation made by others,

Only one new born showed active process of rickets as per skiagram. The calcium and phosphorus contents of the blood of the mother and also the new born were within normal limits. This observation is in contradiction to those recorded in the literature.

Analysis of the mothers' milk.—Analysis of the mothers' milk collected on the fourth day after delivery showed that the average value of calcium was 35.7 mg. with a range varying from 26.5 mg. to 44 mg. per 100 c.c.

The average phosphorus content of milk was 11.2 mg. per 100 c.c. ranging from 6.4 mg. to 16 mg. per 100 c.c. Further work is on progress.

8. Inquiry on the ætiology of toxæmias in the later months of pregnancy under Dr. S. C. Bose and Dr. S. C. Roy at the Medical College, Calcutta.

SYNOPSIS.

The biochemical studies by the Warburg manometric technique indicated that placenta is a rich source of mono-amino oxidase and the total oxygen uptake by the placental tissue from the toxæmic patient is lower than that from normal patient. From the measurement of oxygen-absorption capacity by the manometric gas-analysis apparatus of van Slyke it was observed that the functional hæmoglobin in the maternal venous blood corresponds with that by the Hellige's hæmometer, while certain differences were, however, observed in the case of foetal blood in the normal late pregnancy.

The incidence of toxæmia has been found most common in the two extreme ends of child-bearing age, particularly in the later months of first pregnancy, and the degree of hypertension has got a definite bearing on the other manifestations of toxæmia.

The placental reserve capacity was found to be definitely lower in the toxæmia group as judged by the histological study of placental infarcts.

BIOCHEMICAL.

(a) The oxygen-absorption capacity of blood from normal pregnant women during different stages of foetal development was measured by the manometric gas-analysis apparatus of van Slyke. Blood was drawn from peripheral vein (usually from any anti-cubital vein) and oxalated properly (1 mg. of potassium oxalate per c.c.). The results of volume per cent oxygen capacity were expressed in terms of hæmoglobin (g. per 100 c.c.) by multiplying the volumes per cent with 0.736 (1 g. of hæmoglobin when fully saturated with oxygen contains 1.34 c.c. of oxygen, i.e. each c.c. of oxygen is contained in 0.736 g. of hæmoglobin). The hæmoglobin values as determined by oxygen-absorption capacity were compared with the values as obtained from Hellige hæmometer. This gives the actual amount of functional hæmoglobin circulating in the particular condition,

Hæmoglobin levels and oxygen capacity in peripheral venous blood in normal late pregnancy.

Weeks of pregnancy	Number of cases	HÆMOGLOBIN ESTIMATED ACCORDING TO HELBIG'S METHOD (G. PER 100 C.C.)		OXYGEN CAPACITY EXPRESSED IN TERMS OF HÆMOGLOBIN (G. PER 100 C.C.)	
		Average	Range	Average	Range
36	7	10·50	10·0–12·50	10·20	9·90–122·0
37-38	8	11·25	10·25–12·50	11·80	10·05–125·0
39-40	6	11·05	9·20–12·90	10·90	10·45–116·0

The amount of functional hæmoglobin circulating in maternal and foetal blood in toxæmia cases is under investigation.

CLINICAL.

Uptil now 42 cases of pregnancy toxæmias have been included in the present study: The nephritic and hypertensive toxæmias were excluded from this study by routine examination of cardiovascular system for enlargement of heart and thickening of radial artery, urine for specific gravity (values less than 1,010 rejected), urea concentration (values less than 2·5 per cent rejected), urine for granular or epithelial cast, and fundus oculi by ophthalmoscope to visualize directly for any organic changes in the vessels of retina (cases showing arteriosclerotic changes were rejected).

For the convenience of the present investigation, the patients have been divided in three main groups : (1) Only hypertension in pregnancy, (2) hypertension and albumin urea in pregnancy, and (3) hypertension, albumin urea and/or œdema in pregnancy. Out of each group, study is being carried out on :—

I. Incidence of toxæmias.:—

- (a) in different age groups,
- (b) according to parity, and
- (c) according to period of gestation.

II. Relation between degree of hypertension and severity of toxæmias :—

- (a) hypertension and œdema,
- (b) hypertension and albumin urea,
- (c) headache, visual disturbances and hypertension, and
- (d) hypertension and foetal death.

III. Relation between the rate of fall or rise of blood pressure and other symptoms to different lines of treatment :—

The patients were again arranged into various groups according to the different lines of treatment adopted, viz. (a) salt-free diet, (b) sedative (ranging from simple bromide mixture to morphine and even sometimes

sympatholytic drugs, such as hexamethonium compound according to severity and height of blood pressure, (c) amino acids, (d) iron, and (e) multivitamins—all these in various combinations.

HISTOLOGICAL.

The placentas of toxæmia cases were examined both microscopically and macroscopically after fixing in formol saline solution and also corroborated by histological examination for doubtful areas.

FILARIASIS

1. Filariasis control unit (Orissa) under the Director, Malaria Institute of India, Delhi.

The work reported last year was continued in the four selected areas, namely, the anti-larval, anti-adult, mass-therapy and the comparison areas. The indices for assessment of results continued to be the same, i.e.

1. The microfilarial rates, determined in intervals of 4, 14, 24 and 36 months after the initial surveys, in comparison, anti-adult and mass-therapy areas and 4, 18, 30 and 42 months in the anti-larval area.

2. Vector density (bi-weekly collection).

3. Infection rates in the vector species.

4. Morbidity rates, as ascertained by sample clinical surveys, number of attacks of filarial fever, lymphadenitis and lymphœdema that the persons suffered from.

5. Estimation of DDT residual deposits on the sprayed surfaces by Alessandrini's method.

Anti-larval area.—One per cent DDT-malariol solution was applied at the rate of approximately four gallons per acre of water surface once a week in the suburbs of Cuttack.

Anti-adult measures.—Four applications of residual spraying (in-door) of DDT-aromax emulsion at the rate of 100 mg./sq. ft. were made in Jeypore and part of Chandanpur in Sakhigopal, Puri district and a 200 mg./sq. ft. dose was sprayed four times in another part of Chandanpur.

Mass-therapy area.—Observations of microfilarial rate and the other indices were continued, but no drug was repeated subsequent to one carried out in 1950.

RESULTS.

In the anti-larval area the density of mosquitoes was reduced by about 80 per cent of pre-control levels.

Seasonal fluctuations were noted in the density and infection rates in the mosquitoes collected from the comparison and mass-therapy areas. In the latter the infection rates in the mosquitoes in some months was as high as in the comparison area showing that active transmission was going on.

The "clinical inquiries" revealed that in the anti-larval and anti-adult areas, the average number of attacks of filarial fever was about the same as was observed last year, which was lower than the pre-control level. In the comparison areas the average number of attacks per person progressively increased. In the mass-therapy area, however, while there was an increase in the number of attacks in 1950 (due to the reaction of hetrazan in persons with *microfilaria* the) same have now become lesser than the pre-control level and the clinical improvement noted in some persons who were on therapy has been maintained in a great measure.

From the results of the blood surveys, the trends of infection in persons examined in common at various surveys, in the different areas (expressed as per cent) are as follows :—

TABLE I.

Trends in infection rates in persons examined in common at various surveys as compared to pre-control level.

Area	Surveys			
	I and II	I and III	I and IV	I and V
Mass therapy area (community as a whole) ...	41·6	44·1	50·1	62·6
Imagocidal ...	112·1	135·0	117·7	100·0
Larvicidal ...	110·2	97·3	77·8	76·66
Comparison ...	133·2	114·3	145·1	158·3

From the above, it could be stated that in the comparison area there is no natural decline of infection to date as compared to pre-control level. In the anti-larval area the steady reduction obtained during the first four surveys has not been maintained during the latest survey. After nearly three years of imagocidal control the infection in the community after an initial rise has progressively diminished to its original level only.

In the mass-therapy area, however, the infection rate has progressively risen and in the recent survey markedly so.

In addition, the progress of infection in persons in the mass-therapy area who were initially negative but had therapy, were followed up for possible infections. The percentages of such persons becoming positive at the different surveys were 1·4, 1·9, 1·4 and 3·4, respectively. Contrasted to this, those persons examined in common and who were initially negative and did not take therapy, 11 to 23 per cent became positive. As the numbers of persons examined in this category were small, these figures are not a true sample. The figures for similar persons in the comparison area or those where the parasite had not been interfered with (as in the imagocidal area) always had shown over five per cent of such persons becoming positive. Thus, it could be stated that while there was a gradual rise in such persons who were on therapy and were initially negative, in the present survey, there has been an increase in the number of such persons. Added to this the fact that of the five, who became positive in the present survey, three of them had a full course of therapy under strict supervision. This fact and the rise of infection rate during this year are significant.

An analysis of persons examined in common who were positive initially in the recent survey showed that of 19 persons who initially had a count below 10 mf./20 cmm. three increased to infective levels.

Nearly 50 per cent of the children in the age group of two to five (refers to their age in 1950) in Cuttack suburbs were examined and no fresh infections were noted. Children of the age of two at that time were

examined and found negative at successive surveys. In the other area, it was possible to examine 30 to 35 per cent of children of the same age group with similar results.

At present, from the trends of infection in the various communities and negative persons becoming positive and vice versa, the following preliminary conclusions may be drawn from the results obtained: —

1. Anti-larval measures carried out for 42 months have produced some reduction in the resultant infection.
2. Anti-adult measures carried out for 36 months has brought the infection to the pre-control level which indicates that fresh transmission has been little or negligible.
3. That mass therapy with hetrazan lasting for five days reduced the microfilarial rate dramatically and the reduction has been progressively lesser. During the current survey the infection rate showed an increase.

The rise in the infection rate in the recent survey in the mass-therapy area coupled with the fact that a number of persons who had received therapy and were negative initially became positive are probably pointers that the turning point in the efficacy of the drug has been reached. Added to the above is the auxiliary point of the group of persons positive for *microfilariae* with counts below 10 mf. in 20 cmm. of blood at the pre-control survey now reaching infective levels to mosquitoes. Thus, it would be desirable to follow up the results in this area for at least another survey in April to July 1954, to be more definite as to the happenings in that community. At the same time, it is necessary to examine as many children as possible, especially those who were aged two years in 1950.

2. Filariasis inquiry under the Director, King Institute, Guindy, Madras.

Nine hundred and sixty lizards were brought of which 167 showed *microfilaria*.

TABLE.
Showing Conspicuum infection rates in Calotes.

Year	Number of Calotes examined	Number infected	Percentage infected
1947	653	252	38.6
1948	2,811	961	34.2
1949	2,315	677	29.2
1950	N o t e x a m i n e d		
1951	802	189	23.6
1952	1,509	310	20.54
1953 up to end of September	648	112	17.3

A good number of male and female filarial worms were collected from lizards,

3. Inquiry on pharmacological and toxicological studies of compounds prepared for chemotherapy of filariasis under Dr. G. Werner at the School of Tropical Medicine, Calcutta.

Tables I and II give a complete record of the results of acute and chronic toxicity tests carried out so far with compounds made available to the inquiry.

All substances were used in aqueous solutions ; the results of acute toxicity tests represent averages over at least three to five individual observations. The acute effects in anæsthetized rabbits (urethane anæsthesia, 1.5 g./kg., intravenous) and cats (chloralose, 0.08 g./kg., intravenous) were also observed repeatedly (at least three times) to exclude chance effects.

Histological specimens were only taken from such chronic intoxicated animals which were killed immediately before dissecting out of the specimens to avoid autolysis.

The data on *in vitro* anti-filaria activity (Dr. N. V. Bhaduri, Indian Council of Medical Research reports 1949-52) are added in the tables to show which compounds appear promising for further work.

By that means it can be seen that compounds Nos. 4, 7, 10 and possibly No. 11 offer a comparatively good relation between *in vitro* anti-filaria activity and chronic as well as acute toxicity.

TABLE I.

Substances of which acute and chronic toxicity and acute pharmacodynamic actions have been done.

Number	Substances	Acute I.V. toxicity in rats (dose in mg./100 g.)	Acute effects in anaesthetized cat or rabbit (dose in mg./kg.)	Chronic toxicity in rats (dose in mg./100 g.)	Histological findings in following chronic administration	Remarks	Remarks (<i>in vitro</i> anti-filaria) cf. I.C.M.R. reports 1949-52
1.	Disodium salt of 3 : 3 di-hydroxy azo- benzene-4 : 4-carboxylic acid	10 mg. ; death from respiratory paralysis in approximately 10 minutes.	Rabbit : up to 70 mg. no effect on blood pressure, respiration and circulatory reflexes.	(a) 5 mg. (i.v. for 9 consecutive days); no death. (b) 1 mg. (i.v. for 10 consecutive days) ; no death.	<i>In a and b :</i> <i>Adrenal gland.</i> —large area of necrosis in the adrenal cortex. Loss of structure of fasciculata, while remainder of the tissue exhibits signs of hypertrophy, fibrinoid degeneration. Hgs in some places. <i>Liver.</i> —Areas with centrilobular necrosis ; signs of toxic degeneration of the parenchyma. <i>Kidney.</i> —Focal inflammatory changes seen.	...	1 : 8000 (16 hrs.)
2.	Astrafloxine FF	2.5 mg. ; death in 15 minutes from respiratory paralysis.	Cat : 20 mg. causes marked fall of B. P. and respiratory arrest. 10 mg. and less ; transient fall of B.P. followed by short-lasting rise.	(a) 1 mg. ; death after 2 i.v. on 2 consecutive days (b) 0.5 mg. ; death after 3 i.v. on 3 consecutive days (c) 0.25 mg. ; i.v. for 6 consecutive days ; no death.	<i>In c :</i> (killed after 6 injections of 0.25 mg. each) ; extreme vasodilatation in all the parenchymatous organs and multiple extravasations.	...	1 : 64,000 (17 hrs.)

TABLE I (contd.)

Number.	Substance	Acute I.V. toxicity in rats (dose in mg./100 g.)	Acute effects in anaesthetized cat or rabbit (dose in mg./kg.)	Chronic toxicity test in rats (dose mg./100 g.)	Histological findings in rats following chronic administration	Remarks	Remarks (<i>in vitro</i> antilaria. of I.C.M.R. reports 1949-52)
3.	Sulphanilamide salt with naphthalenesulphonic acid.	With 10 mg.; no death.	Rabbit : up to 30 mg.; no effect on B.P. and respiration.	(a) With 5 mg. ; for 9 consecutive days ; no death. (b) 10 mg. for 3 consecutive days no death.	<i>In a :</i> <i>Liver.</i> —Slight indication of beginning centrilobular necrosis. <i>Adrenal gland.</i> —Necrosis over large areas of the zona fasciculata. <i>In b₁ :</i> Extensive necrosis of the liver and adrenal glands as in (a).	Intracasternal injection of 2.5 mg./1 kg. causes tremor, hyperventilation and convulsions (suppressed by Myanesin i.v.)	1 : 1,28,000 (22 hours.)
4.	Sulphanilamide salt with phenol-p-sulphonic acid.	With 20 mg.; no death.	Cat : 70 mg. (in the course of 30 minutes) ; no effect on B. P. and respiration.	(a) 10 mg. (i.v. for 6 consecutive days, followed by i.p. for 2 consecutive days) ; no death. (b) 20 mg. (i.v. for 2 consecutive days ; followed by i.p. 1 day) ; no death.	<i>In a and b :</i> <i>Adrenal gland.</i> —Vacuolization of the cells of zona fasciculata ; characteristic picture of mild stress. <i>Liver.</i> —Severe fatty vacuolization.		1 : 1,28,000 (22 hours).
5.	4-acetamido-2-methoxy-stibonic acid	With 5 mg. ; no death.	Cat : 2.5 mg. to 5 mg.; transient fall of B.P. Total dose of 35 mg. ; no prolonged effect on mean arterial pressure and spontaneous respiration.	5 mg. (i.v. for 2 consecutive days followed by i.p. for 2 consecutive days) ; no death.	<i>Liver.</i> —Massive necrosis.	At the site of injection. Severe gangrene ; following i.p. inj : peritonitis and liver abscess.	

TABLE I (contd.)

Number	Substance	Acute I.V. toxicity in rats. (dose in mg./100 g.)	Acute effects in anæsthetized cat or rabbit (dose in mg./kg.)	Chronic toxicity test in rats (dose in mg./100 g.)	Histological findings in rats following chronic administration	Remarks	Remarks (<i>in vitro</i> anti-filaria) cf. I.C.M.R. reports 1949-52
6.	Propylester of p-carboxystibonic acid.	10 mg. (i.p.) ; death within 24 hours.	<i>Adrenal gland.</i> —The entire fasciculata assumed spongy appearance with the formation of many lumina due to disintegration and casting off cortical cells. It is no longer possible to differentiate between lumina and sinusoids since none of the visible channels is filled with normal blood cells. Such adrenals give the impression of a holocrine secretion of cortical cells into the lumina, which also receive blood from ruptured sinusoids. In the channels so formed, both cortical and blood cells disintegrate, their remnants being flushed down towards the medullary sinusoids.	24 hours after single injection adrenal gland shows the histological findings. No substance left.	1 : 1,28,000 (15 hours).
7.	4-laurylamido-2-methoxystibonic acid.	8 mg.; no death.	Rabbit : 10 mg. causes marked transient fall of B.P. and prolonged inhibition of carotid-sinus reflexes.	(a) 8 mg. (1 day i.v. followed by 1 day i.p.); death on the 3rd day. (b) 3 mg. i.v. for 3 consecutive days ; death on the 4th day. (c) 1 mg. i.v. for 6 consecutive days ; no death.	<i>In c :</i> No significant changes found.	...	1 : 1,60,000 (14 hours).

TABLE I (contd.)

Number.	Substance	Acute I.V. toxicity in rats (dose in mg./100 g.)	Acute effects in anæsthetized cat or rabbit (dose in mg./kg.)	Chronic toxicity test in rats (dose in mg./100 g.)	Histological findings in rats following chronic administration	Remarks	Remarks (<i>in vitro</i> anti-filaria) cf. I.C.M.R. reports 1949-52
8.	p-carboxystibonic acid.	5 mg.; no death.	Cat : 1 mg. without marked effect; 5 mg. causes marked fall of pressure with short lasting arrest of spon-respiration.	(a) 2.5 mg. i.v. for 8 consecutive days; no death. (b) 5 mg. i.v. for 3 consecutive days; no death.	<i>In a and b :</i> <i>Liver.</i> —Dilatation of the sinusoides. No other changes found in any other organs.	At the site of injection (tail) ; swelling, necrosis and spreading gangrene.	1 : 64,000 (16 hours).
9.	p-laurylamidostibonic acid.	10 mg.; immediate death (rigor, fasciculation). 5 mg.; death in 3 to 5 minutes.	Cat : Up to 15 mg.; only slight fall of B.P.	(a) 2.5 mg. i.v. for 3 consecutive days; death. (b) 1 mg. i.v. for 10 consecutive days; no death.	<i>In b :</i> <i>Liver.</i> —Granulation of the cytoplasm of liver cells. <i>Kidney.</i> —Focal nephritis.	At the site of injection (tail) ; spreading gangrene found.	...
10.	p-acetamidostibonic acid.	15 mg.; death within 7 minutes. 5 mg.; death in 25 minutes (respiratory paralysis).	Cat : 2.5 mg. causes short-lasting steep fall of B.P.	(a) 2.5 mg. i.v. for 2 consecutive days; death. (b) 1 mg. i.v. for 10 consecutive days; no death.	<i>In b :</i> <i>Kidney.</i> —Round cell infiltration in patches glomeruli destroyed and replaced by fibrous tissue. Fibrinoid material in tubuli, tubular epithelium lost and degenerate in a few places. Hemorrhages seen in places. <i>Other organs.</i> —No change.	...	1 : 1,640,000 (12 hours).

TABLE I (contd).

Number	Substance	Acute I. V. toxicity in rats. (dose in mg./100 g.)	Acute effects in anaesthetized cat or rabbit (dose in mg./kg.)	Chronic toxicity test in rats (dose in mg./100 g.)	Histological findings in rats following chronic administration	Remarks	Remarks (<i>in vitro</i> anti-filaria) cf. I.C.M.R. reports 1949-52
11.	p-anisidine anti-monyl tartrate.	10 mg.; death within 6 hours. 5 mg. i.p.; death within 16 hours.	Cat: Single doses up to 15 mg. without effect on B.P. and respiration; after 50 mg. (within 1 hour) death from arrest of heart.	2 mg. i.v. for 6 consecutive days; no death.	<i>Adrenal gland</i> .—Haemorrhages seen in the cortex and also subcapsular. <i>Other glands</i> .—No change.		1 : 122,000 (16 hours).
12.	Salt of Nekal BX acid with sulphamamide soluble.	5 mg.; no death.	Cat: 1 mg. to 5 mg.; short lasting biphasic pressor - depressor change of B.P. After 40 mg.; respiration stopped.	5 mg. i.v. for 3 consecutive days (death on the 4th day).	No changes found.	No substance left.	1 : 8,000 (23 hours).
13.	Salt of naphthalene - beta - sulphonic acid with sulphathiazole.	5 mg.; no death.	Cat: 1 mg. to 5 mg.; short biphasic pressor-depressor effect on B.P. 15 mg.; increase of depth and frequency of respiration. Total dose of 60 mg.; survived.	5 mg. i.v. for 3 consecutive days (death on the 4th day).	<i>Liver</i> .—Passive congestion seen.	No substance left.	1 : 8,000 (22 hours).

Substances of which acute and chronic toxicity and acute pharmacodynamic actions have been done (still in progress).

No.	Substance	Acute I.V. toxicity in rats (dose in mg./100 g.)	Chronic toxicity test in rats (dose in mg./100 g.)	Acute effects in anaesthetized cat or rabbit (dose in mg./kg.)	Remarks <i>in vitro</i> antilaria action I.C.M.R.- reports 1949-52
1.	p-phenetidine anti- monyl tartrate.	10 mg.; death within 7 hours. 5 mg.; death within 12 hours.	1 mg. i.v. for 10 conse- cutive days; no death.	With 2.5 mg. to 10 mg. causes fall of B.P. with total 40 mg. (1½ hours); death.	1 : 40,000 (21 hours).
2.	Methyl-p-amino- benzoate antimonyl tartrate.	With 10 mg.; death within 6 hours. 5 mg.; death within 18 hours.	1 mg. i.v. for 10 conse- cutive days; no death.	1 mg. i.v. causes fall of B.P. Total dose of 48 mg. within 1 hour; survived; no change of respiration and C.S.R.	1 : 256,000 (16 hours).
3.	Ethyl-p-amino-ben- zoate anti-monyl tartrate.	10 mg.; death within 10 minutes. 5 mg.; death within 18 hours.	2.5 mg. i.v. for 2 conse- cutive days followed by 1 i.p.; no death.	With a single dose of 2.5 mg.; fall of B.P. about 25 mm. of Hg. Total dose of 25 mg.; only fall of B.P. No change of C.S.R. or respiration.	1 : 256,000 (16 hours).
4.	2-aminobiazol anti- monyl tartrate.	10 mg.; death within 15 minutes. 5 mg.; death within 16 hours.	1 mg. i.v. for 10 conse- cutive days; no death.		1 : 128,000 (17 hours).
5.	Sodium beta naph- thalene sulphonate.	5 mg.; no death.	(a) 5 mg. for 3 conse- cutive days i.v. died on the 4th day. (b) 2.5 mg. i.v. for 9 consecutive days; no death.	2.5 mg. to 20 mg. injection pressor-depressor effect. Total dose of about 70 mg.; no other changes found.	1 : 16,000 (20 hours).
6.	Salt of naphthalene- beta-sulphonic acid with sulpha-pyridine, soluble.	10 mg.; no death.	(a) 10 mg. for 6 consecutive days i., no death. (b) 5 mg. i.v. for 10 consecu- tive days; no death.	With single doses of 2.5 mg. to 10 mg. causes fall of B.P. Total dose of 68 mg. within 1½ hours death. No effect on C.S.R. or respiration.	1 : 8,000 (22 hours).
7.	Propyl-p-amino-ben- zoate antimonyl tar- trate.			With single doses varying from 2.5 mg. to 20 mg. causes pressor-depressor effect. Total dose of 65 mg. within 1½ hours causes death from circulatory failure.	1 : 128,000 (16 hours.)

N.B.—Histological works are in progress.

VIRUS DISEASES

1. Rabies research under the Director, Central Research Institute, Kasauli.

I. The following report deals with the results of trials of hyper-immune antirabic serum used for the treatment of street virus infection in experimental animals :—

Ninety guinea-pigs of 500 g. to 600 g. weight were injected intramuscularly in the leg muscles with 1 c.c. of street virus infected guinea-pig brain—1/50 dilution in distilled water. The animals were divided into six groups of 15 each and treated one hour later.

Period of observation ... Ninety days.

The results are shown in Table I :—

TABLE I.

Street virus, intramuscularly, followed by various types of treatment.

Group	Treatment	Deaths/ total	Per cent deaths	Period to death, days	Average period to death, days
1.	Serum 1 c.c. I.P. ...	10/14	72	30, 36, 37, 42, 48, 48, 50, 54, 56, 67,	47
2.	Five per cent vaccine six doses of 1 c.c. each I.P. ...	3/15	20	12, 12, 31	18
3.	Twenty per cent vaccine single dose of 1.5 c.c. I.P.	3/15	20	18, 28, 50	32
4.	Serum 1 c.c. and five per cent vaccine six doses of 1 c.c. I.P.	3/15	20	37, 37, 43	39
5.	Serum 1 c.c. and 20 per cent vaccine 1 dose of 1.5 c.c.	8/12	67	18, 21, 22, 32, 34, 43, 47, 49	33
6.	Untreated controls ...	11/15	73	17, 18, 18, 19, 20, 21, 24, 24, 25, 29, 32	22

Note :— 1. The vaccines used were phenolized Semple's type of antirabic vaccines.
2. The serum used was an ammonium-sulphate purified fraction capable of neutralizing 10⁶ Mouse LD 50s of fixed virus.

Comments.—Attention is drawn to (1) the prolongation of the incubation period of rabies in experimental animals treated with immune serum, (2) results so far as survival is concerned are disappointing (*see* group I), (3) serum in conjunction with a course of vaccine gives good results, which are, however, no better than with the course of vaccine alone—group II and IV refer, and (4) a notable feature is the blanketing effect of the serum when a single dose of 20 per cent vaccine is given simultaneously.

These results confirm our findings (1951) regarding the use of antirabic serum in conjunction with vaccine treatment.

II. *The comparative efficiency of different forms of local treatment of wounds exposed to street virus infection.*—To test the efficacy of different forms of local treatment of wounds in preventing rabies, studies were undertaken in monkeys and guinea-pigs, using street-virus salivary-gland suspension on brain-cum-hyaluronidase, and treating the wounds 25 minutes after infection. The method adopted was to inflict a wound in the gluteal region and then contaminate it with street virus. The shaved skin over the back of the thigh was incised, either with a razor blade or with scissors, some of the muscle fibres cut and rubbed with a pledget of cotton-wool soaked in ten per cent street-virus suspension. One of the chief difficulties encountered was that many animals died of post-traumatic secondary infection, the wound in some animals showed sloughing and gangrene, particularly in the case of those treated with nitric and carbolic acids. Of the 70 animals treated in one series, 31 died within the first 15 days and many others died subsequently of causes other than rabies. This method of infection was, therefore, given up and efforts directed to perfect a technique which would cause the least amount of trauma and at the same time ensure rabic infection and allow subsequent local treatment of the wound. A special punch imitating a terrier's mouth has been devised, the tooth in the head of the punch is patent, like the fangs of a snake, through which the infective material can be forced into the tissues, while the punch is *in situ*. The virus is thus allowed to come in contact with the tissues for two to three minutes before the punch is disengaged.

Another method employed was to use a surgical needle threaded with a four-ply strand of wool soaked in 20 per cent street-virus suspension and passed through the skin and muscles of the thigh of guinea-pigs. A measured length of the infected thread is left *in situ* for 20 minutes. This is then pulled out and the succeeding length of the strand, soaked in the cauterizing agent under test, is allowed to come into contact with the tissues of the infected tract for a period of two to three minutes.

The third method which has given very satisfactory results is the use of a rotary vaccinating lancet (five teeth) soaked in 20 per cent street-virus suspension and used for infecting the gluteal muscle exposed by a skin incision. The results are given in Table II:—

TABLE II.

Local treatment of wounds produced by the vaccinating lancet and exposed to rabic infection.

Group	Treatment	Number of guinea-pigs	Deaths	Per cent deaths
I.	Pure carbolic acid	10	5	50
II.	Fuming nitric acid	10	4	40
III.	Pot. permanganate (1/1,000 D.W.)	10	5	50
IV.	Soap solution, one per cent	10	5	50
V.	Untreated controls	10	8	80

Comments.—It would appear that the use of strong cauterizing agents, such as carbolic or fuming nitric acids, has no advantage over the applications of non-caustic agents, such as potassium permanganate or common or garden soap solution, provided the latter are used freely and reach the depths of all pockets. Irrigation with soap solution or potassium permanganate not only helps to wash away infective material but recent work carried out in experimental animals shows that these agents are viricidal in high dilutions. In the light of these findings, the orthodox treatment of persons bitten by animals suspected to be rabid, using strong cauterizing agents as a first-aid measure will require revision if the results are confirmed in a larger series of animals.

III. *Effect of light on potency of antirabic vaccine.*—A great deal of work has recently been carried out on the action of sunlight on the antigenicity of bacterial vaccines, particularly B.C.G. vaccine, and it has been shown that it is not so much the effect of high temperature which is responsible for the deterioration of these vaccines as the action of light. It was, therefore, considered worthwhile investigating the effect of sunlight on the potency of antirabic vaccine which has also a limited life. The experiments are in progress.

IV. The use of mucin as an adjuvant to increase the immunizing efficacy of antirabic vaccine was tried in animals previously injected with street virus.

The results showed that it did not accelerate the development of immunity or increase the protective value of the vaccine.

V. *Adaptation of local strain of rabies street virus to chick embryos.*—Four locally isolated strains of street virus were serially passaged in chick embryos in an attempt to adopt this method of culture. The study is in progress.

VI. *Potassium permanganate as an inactivating agent in production of antirabic vaccine.*—Potassium permanganate is commonly used as a topical application in the local treatment of smallpox eruption and is known to be a potent viricidal agent for this virus. An investigation of its rabicidal properties, with a view to its use as an inactivating agent, in place of phenol, in the preparation of antirabic vaccine was undertaken.

A 20-per cent fixed-virus rabbit-brain emulsion was incubated for 48 hours with equal quantities of 1/500, 1/5,000 and 1/50,000 potassium permanganate in distilled water and the mixtures tested in guinea-pigs for evidence of viable virus.

Potassium permanganate in a dilution of 1-1,000 was found to inactivate ten per cent fixed-virus brain emulsion in 48 hours at 37° C. Further work on the antigenicity of potassium permanganate inactivated vaccine is contemplated.

2. Polio research unit under Dr. P. V. Gharpure at the Grant Medical College, Bombay-8.

The report is presented under the following heads :—

- A. Epidemiological studies.
- B. Virus studies.
- C. Extra work done.

A. EPIDEMIOLOGICAL STUDIES.

The report is presented under the three heads :—

1. Clinical work in Bombay.
2. Field work in Bombay.
3. Poliomyelitis in Dohad.

The proforma in use for the collection of information has been revised on the lines of the tabulations received from the All-India Institute of Hygiene & Public Health, Calcutta.

1. *Clinical work.*

One hundred and fifteen cases were studied between 1-9-1952 and 31-8-1953. Of these, 11 were declared non-polio on clinical and other grounds.

(i) The cases were seen at the following centres in Bombay :—

Byramjee Jeejeebhoy Hospital for children	40
Jerbai Wadia Hospital for children	25
King Edward Memorial Hospital	3
Sir Jamshedji Jeejeebhoy Hospital	1
Orthopedic Hospital of the Society for the Rehabilitation of Crippled Children	35
Total			104

The 35 cases shown against the Orthopedic Hospital were convalescent cases ; the rest, viz. 69 were acute.

From the records of the Bombay Municipality it was observed that notification of 67 cases was received for the same period.

Cases going to the Orthopedic Hospital are from all parts of the country, including Bombay. Many of these are likely to have been treated in the home as these are from well-to-do families. Such a group, coming as it does from the whole country, will be useful for collecting bloods for neutralization studies.

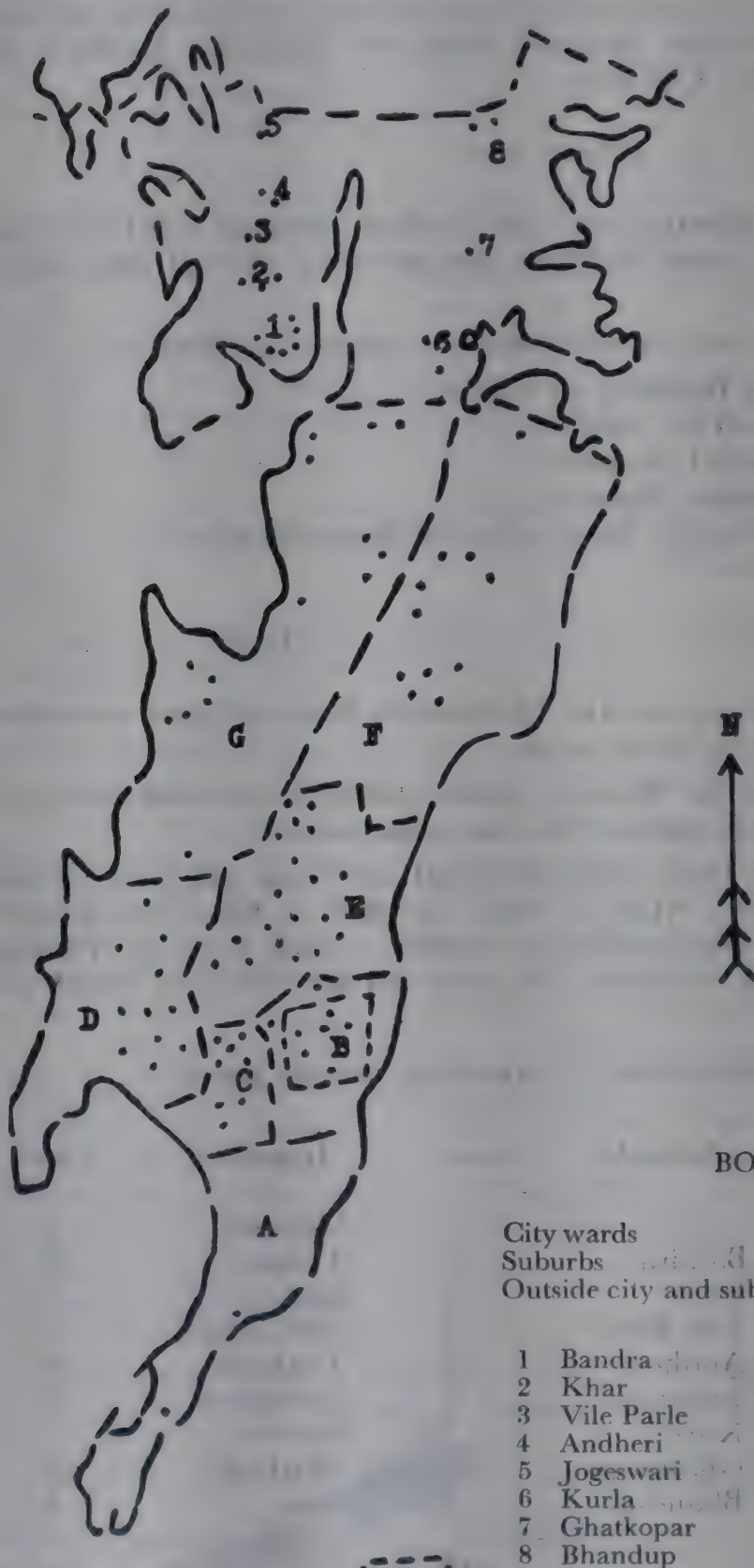
(ii) *Geographical distribution (see spot map, reverse page).—*

City ward	Cases	Suburbs	Cases	Imported	Cases
		Bandra	6	Mulund	1
A	2	Khar	3	Thana	3
B	8	Vile Parle	1	Kalyan	2
C	8	Andheri	2	Ambarnath	2
D	11	Jogeswari	1	Thakurle	1
E	19	Kurla	2	Goregaon	1
F	11	Ghatkopar	1	Borivili	1
G	14	Bhandup	2	Murbad	1
				Goa	1
Totals	73		18		13

Polio in Bombay, suburbs, and near-by areas

(From 1-9-1952 to 31-8-1953)

. 9 (17 miles)	. 12 (21 miles)
. 10 (21 miles)	. 13 (31 miles)
. 11 (19 miles)	. 14 (34 miles)
	. 15 (38 miles)



BOMBAY

City wards	A to G
Suburbs	1 to 8
Outside city and suburbs	9 to 15

1 Bandra	9 Goregaon
2 Khar	10 Borivili
3 Vile Parle	11 Mulund
4 Andheri	12 Thana
5 Jogeswari	13 Thakurle
6 Kurla	14 Kalyan
7 Ghatkopar	15 Ambarnath
8 Bhandup	

Selected area in ward B
 Each dot represents one case
 Figure in brackets is of miles from
 Victoria Terminus.

Steadily more cases are coming from the suburbs and places nearby. Imported cases have come from places within 50 miles from the centre of the town and within about five to ten miles from the suburbs. This feature may be explained on grounds of greater notice being taken of the disease or on grounds of centrifugal spread.

(iii) *Distribution by months.*—

1952			1953		
Month		Cases	Month		Cases
September	...	18	January	...	11
October	...	9	February	...	12
November	...	10	March	...	7
December	...	5	April	...	9
			May	...	5
			June	...	2
			July	...	10
			August	...	6

January and February are the months with the maximum incidence in 1953. The tendency of the maximum occurrence shifting towards the winter months appears to persist. This feature will need very careful study on the lines already in progress, i.e. throat-swabs study and general survey of the population.

(iv) *Age and sex distribution.*—

Age				Males	Females	Total
0-6 months		3	3	6
6-12 months		12	14	26
1-2 years		25	16	41
2-3 years		12	5	17
3-4 years		4	3	7
4-5 years		0	2	2
5-10 years		1	3	4
10-15 years		0	0	0
16 years and above		0	1	1
Totals				57 (55%)	46 (45%)	104

Eighty-four cases are in the age group six months to three years ; five cases in the age-group of above five years and one of these is a female of 26—the case from Goa.

(v) *Distribution of paralysis.*—Paralytic cases had the usual two-stage illness. In 75 per cent cases the leg was affected. There were seven bulbar cases.

(vi) *Fatality.*—There were six deaths—all in the bulbar type.

The above classified and tabulated account is presented on the same lines as all the previous reports.

2. *Field work.*

(i) *Home visits.*—One hundred and thirty-eight visits were made. The visits were used for the study of homes, patients, contacts, and for collecting materials for virus study.

In 33 households with acute cases, 171 persons were surveyed.

House holds surveyed	33
Persons surveyed	171
Adults	113	
Males	57		
Females	56		
Children	58	
Males	25		
Females	33		
Households with illness	10
Persons sick (with cough and cold)	17

It has not been possible to establish any sequence of the cases or any other correlation.

Contact stools collected have not yielded any positive results. Carriers have not been spotted. Twenty-five stools were collected for this study.

Majority of the homes visited has been of families of the lower economic groups with poor hygiene and generally with abundance of flies, mosquitoes, rats, etc.

(ii) *Throat-swab studies.*—A shift in the seasonal incidence having been noted, this study was undertaken to see if the virus could be detected in the throat of persons from the endemic zones. Persons examined included patients and contacts.

(iii) *Sewage work.*—Before and during the monsoon the samples were collected.

(iv) *Rats.*—Rats from the households were obtained with the help of the municipal authorities.

(v) Flies were also collected.

[Further details of (ii), (iii), (iv) and (v) will be found under Virus Studies].

For the search of the reservoir and the extra-human sources of the polio virus (ii, iii, iv, v) the highly infected city ward B with a population of 187,122 (census 1951) and density of 455.3 per acre was selected. The average incidence of polio cases in this area has been 12.83 cases per 100,000 in the last seven years. Majority of the population here is poor, living in small and crowded tenements. The lanes in this area are narrow and lined by rows of back-to-back houses. The selected area includes two schools, a musafirkhana, two temples, three mosques, a jail for delinquent children, and fish and vegetable market. The eastern side of this ward is the dock area with a number of godowns and the local railway line.

3. *Poliomyelitis in Dohad.*

On the 24th of June, 1953, two cases of poliomyelitis in Dohad were reported in the local (Bombay) newspapers. Notice was immediately taken of the information and the Unit was kept in readiness to move a worker to Dohad, if required. Samples of fæces were obtained through the courtesy of the Medical Officer, Municipal Hospital, Dohad, and inoculated in monkeys. A strain of poliomyelitis virus has been isolated from one of the samples received.

B. VIRUS STUDIES.

Under this part are presented results of the following :—

- (1) Isolation of fresh strains,
- (2) Examination of sewage,
- (3) Examination of throat swabs,
- (4) Search for extra-human sources,
- (5) Maintenance of virus strains, and
- (6) Attempts at adaptation to rodents and chick embryos.

1. *Isolation of fresh strains of poliomyelitis virus.*—

(i) *From clinical material from Bombay and its suburbs.*—Stools from 35 clinical cases of poliomyelitis from Byramjee Jeejeebhoy and other hospitals in the city and 25 contacts were available for isolation of the virus. Material from 14 out of the 35 patients was inoculated singly, while the rest was pooled into seven batches. The contact specimens were pooled into three batches.

Three single stools and one pool revealed the presence of poliomyelitis virus. The infective cord material has been passaged successfully through one passage each in the monkey.

It is of interest to note here that one of the strains of the virus isolated is from a case of paralytic poliomyelitis from Goa. The patient, Mrs. F. L., age 26 years, of Bodiem (Goa), had fever, headache and giddiness on 9-8-1953. On 14-8-1953 paralysis of the left arm with tremors of the lower extremities was manifest. She was brought to Bombay and admitted to J. J. Hospital on 27-8-1953. The specimen of stools obtained from her on 2-9-1953 revealed the presence of the virus.

As this is a strain isolated from outside the limits of Bombay State, it is intended to study this strain in detail and compare it with the Bombay strains.

(ii) *From clinical material from Dohad.*—Three specimens of stools were received and inoculated singly into *rhesus* monkeys. One specimen revealed the presence of the virus. This strain has been further passaged.

This is the first successful isolation of a strain of poliomyelitis virus from a case of the disease from Dohad.

(iii) *From stool specimens collected by Dr. D. D. Banker, M.D., of the Seth G. S. Medical College, for isolation of coxsackie viruses.*—Twenty-three specimens were received. After treatment with penicillin and streptomycin, the

specimens were inoculated intracerebrally in rhesus monkeys, 13 singly and the rest pooled into three batches.

Two of the 13 single specimens and one pool revealed the presence of the virus. It might be mentioned here that none of the animals had any untoward reactions following the direct intracerebral inoculation with the stool material.

TABLE I.
Essential features of the strains isolated.

Serial number	Source of virus inoculum (stools)	Route of inoculation	Monkey number	Spinal-cord lesions	Passage in monkey
1.	Female, 1 $\frac{3}{4}$ years, paralytic case ...	IN	M-243	Present	First
2.	Pool No. 104 ...	IN	M-285	Present	First
3.	Female, 26 years, paralytic case ...	IT	M-311	Present	First
4.	Male, 1 $\frac{1}{4}$ years, paralytic case ...	IN	M-283	Present	First
5.	Male, 1 $\frac{3}{4}$ years, paralytic case ...	IT	M-312	Present	First
6.	Female, 1 $\frac{1}{2}$ years, paralytic case ...	IT	M-264	Present	First
7.	Female, 10 months, paralytic case ...	IT	M-277	Present	First
8.	Female 7 $\frac{1}{2}$ months, Male 9 months ; Male, 1 year ; all paralytic cases ...	IT	M-306	Present	First

IN — Intra-nasal ; IT — Intra-thalamic.

3 — From the imported case from Goa.

4 — Material received from Dohad.

6, 7, 8 — Material received from Dr. D.D. Banker.

Dr. Banker has isolated eight strains of coxsackie virus from amongst the 23 samples. One of the samples contains both coxsackie and poliomyelitis viruses. The other specimens which contain poliomyelitis virus are doubtful for the content of coxsackie virus. These are being further tested for its presence by him. His results will be awaited with interest. The simultaneous finding of coxsackie and poliomyelitis viruses in the same specimen of stool is interesting and is in accordance with the observations of Melnick *et al.* (1953).

2. *Examination of sewage for the content of poliomyelitis virus.*—The technique detailed in last year's report was followed. One hundred and twenty samples of sewage were collected from Dongri, Umerkhadi and Mandvi areas of ward B in the pre-monsoon and monsoon months for virus isolation. Six of these were inoculated singly and the rest pooled into

31 lots and inoculated intranasally into monkeys. None of the specimens revealed the presence of poliomyelitis virus. The work is still in progress.

3. *Examination of throat swabs of contacts of poliomyelitis patients for the content of virus.*—One hundred and sixty-six throat swabs from contacts of poliomyelitis patients were pooled into 28 batches. Care was taken as far as possible to pool only the swabs from the contacts of each patient's house. Each pool, after treatment with penicillin and streptomycin, was inoculated intracerebrally into *rhesus* monkeys. None of the pools revealed the presence of virus.

4. *Search for extra-human sources of virus.*—

(i) *Flies.*—These were collected from the endemic areas in ward B and examined for the content of poliomyelitis virus. Four batches of 2,000 flies each were collected and pooled into two lots. Saline emulsions of each lot was centrifuged at 3,000 r.p.m. for 30 minutes, the supernate treated with ether and kept in the cold for 24 hours. They were then re-centrifuged, the middle aqueous layer pipetted off, treated with penicillin and streptomycin, and inoculated intracerebrally into monkeys. Both the pools did not show the presence of poliomyelitis virus.

(ii) *House rats.*—The intestinal contents of 75 rats collected from the houses of poliomyelitis patients were pooled into ten lots and inoculated intranasally into monkeys. None of the lots revealed the presence of virus.

The above account (paras 2, 3, 4) of negative findings is intended to state what exactly was done. The negative results are not meant to convey the absence of the virus in the material examined. The negative results reported now may be due to technical difficulties. It is felt that this Unit must expand with more equipment and more men to enable it to pursue these problems.

5. *Maintenance of strains of poliomyelitis virus isolated in the previous years.*—The five strains isolated during the previous years are being maintained by passage in *rhesus* monkeys.

The material from monkey No. M-10, the first strain of the virus isolated in this Unit, is potent after storage for 41 months in 50 per cent glycerine under ordinary conditions of refrigeration.

Table II gives the number of passages which each strain has been put through :—

TABLE II.

Strain of virus	Passage history	Material tested after, months
M-10	13th	41
M-38	3rd	19
M-52	8th	25
M-150	2nd	15
M-158	3rd	15

Materials of all these five strains have been sent to Dr. David Bodian, M.D., Poliomyelitis Research Laboratory, The Johns Hopkins University, Baltimore, U.S.A., for further study. The strains, M-10, M-38 and M-52,

have been established in tissue cultures in that Laboratory and have been typed. Strains M-10 and M-52 belong to type I (Brunhilde) and the strain M-38 belong to type II (Lansing).

6. *Attempts at adaptation of the recently isolated strains to rodents and chick embryos.*—Mice and rats were inoculated by the intracerebral and intraspinal routes with the infective material. Five serial blind passages were carried out in mice and rats with no clinical and histological features of the disease.

Seven-day old chick embryos were inoculated with infective monkey-cord suspension by the allantoic and yolk-sac routes. Five serial passages were done and the material from the fifth passage when inoculated intracerebrally into *rhesus* monkey did not produce the disease. The work is still in progress to ascertain if the strains could be adapted by continued and prolonged passage into the chick embryos.

C. EXTRA WORK DONE.

1. *Hæmagglutination studies.*—As was indicated in last year's report certain modifications were made to adapt the Hirst's reaction to the poliomyelitis virus suspension, with no success. These include : (i) Changing the pH of the medium for the preparation of the virus suspension ; (ii) effect of the diluents of different pH employed for the serial dilution of the virus suspension for the test ; and (iii) the effect of pH on the cell-virus reaction system. Further modifications of this reaction are under test.

2. *Influenza virus work.*—Thirty-six samples of throat washings were available for the isolation of virus. All were tested by the standard technique in 13-day old developing chick embryos. Two of the 36 samples revealed the presence of the virus after three amniotic passages in the chick embryos. The material from these two isolations are being sent to Dr. I. G. K. Menon, Pasteur Institute, Coonoor, for further studies.

3. **Inquiry on the antigenic analysis of Indian strains of influenza virus under Dr. I. G. K. Menon at the Pasteur Institute of Southern India, Coonoor.**

Twenty-three strains of influenza viruses have so far been taken up for analysis during the inquiry. Details regarding them are given below:—

Foreign strains kindly supplied by the World Influenza Centre, London.

1. A/PR8
2. A'/FM1
3. A'/Sweden
4. A'/Liverpool
5. A'/Lepine
6. B/Lee
7. B/Crawley
8. B/Paddington

Indian strains.

Strain number :		Date of isolation :	Isolated by :	
9.	Strain 1	5th August, 1950	Menon, Coonoor	
10.	„ 2	14th August, 1950	Menon, Coonoor.	
11.	„ 3	18th August, 1950	Menon, Coonoor.	
12.	„ 4	24th March, 1951	Menon, Coonoor.	
13.	„ 5	— March, 1951	Swaminath, Polio Research Unit, Bombay.	
14.	„ 6	13th November, 1951	Menon, Coonoor.	
15.	„ 7	13th November, 1951	Menon, Coonoor.	
16.	„ 8	21st November, 1951	Menon, Coonoor.	
17.	„ 9	— March, 1952	Lt.-Col. S. L. Kalra, A.F.M.C., Poona.	
18.	„ 10	12th December, 1952	Nalini.	} Influenza Inquiry, Coonoor.
19.	„ 11	20th December, 1952	Murthy.	
20.	„ 12	20th December, 1952	Murthy & Nalini.	
21.	„ 13	24th December, 1952	Joghee.	
22.	„ 14	Received March, 1953	Lt.-Col. S. L. Kalra, A.F.M.C., Poona.	
23.	„ 15	„ „ „ „ „ „ „ „ „ „		

Two methods are available for antigenic analysis of influenza virus strains: the hæmagglutination-inhibition test and the complement-fixation test. The former is more commonly in use, employing infected allantoic fluid as antigen and sera either from relatively susceptible animals, such as the ferret, white mouse and hamster, after intra-nasal inoculation or from insusceptible animals, such as the rabbit and guinea-pig after intravenous or intraperitoneal immunization. The complement-fixation test is carried out with particulate antigens from infected allantoic fluids and human sera. The hæmagglutination-inhibition method was adopted for the inquiry. A few experiments were, however, carried out with a modified complement-fixation test.

The technique recommended by the World Influenza Centre was generally followed, but several important modifications had to be made to suit local conditions. Thus, the non-availability of the ferret and the hamster in the country ruled out their use. The mouse, guinea-pig, rabbit and fowl were alternative sources of sera. Previous workers had pointed out that normal sera from all the four contained inhibitors which had to be removed by treatment with cholera filtrate. The fowl and the rabbit were selected for study in the first instance.

Normal sera from several fowls and rabbits were collected and after de-activation at 56°C. for 30 minutes, tested for inhibitor activity against 17 antigens including eight standard foreign strains, viz. PR8, FM1, Sweden, Liverpool, Lepine, Lee Crawley and Paddington and nine strains isolated in India in 1950, 1951 and 1952. Pooled infected allantoic

fluids were used as antigens. The rabbit sera were found to inhibit them to varying titres (Table I), e.g. 8 or < 8 with PR8 and after PRB strain five, 32 to 128 with Lee, Crawley, Paddington and strains four and six and 256 with FM1, Sweden, Liverpool, Lepine and the remaining six Indian strains, viz. 1, 2, 3, 7, 8 and 9. In sharp contrast, the fowl sera individually as well as in pools from eight birds gave inhibition titres of 8 or < 8 against all the antigens with the exception of Sweden, Liverpool and Indian strain 2, which were inhibited in dilutions of 16. The fowl was accordingly selected for the preparation of anti-sera.

TABLE I.

Non-specific inhibitor content of sera from normal fowls and rabbits against 17 antigens.

Antigens	Fowls	Rabbit
PR8	< 8	< 8
FM1	8	256
Sweden	16	256
Liverpool	16	256
Lepine	8	256
Lee	< 8	64
Crawley	< 8	128
Paddington	< 8	128
Strain 1	8	256
„ 2	16	256
„ 3	< 8	256
„ 4	< 8	32
„ 5	< 8	8
„ 6	8	128
„ 7	8	256
„ 8	8	256
„ 9	< 8	256

The next problem that arose was the optimum method of immunization of the birds. Hilleman, Mason and Rogers (1950) gave combined intravenous and intraperitoneal inoculations on the same day of 5.0 c.c. amounts of infected allantoic fluids with hæmagglutinating titres of 320 or more and collected the sera after ten days. Three birds were immunized against PR8, Liverpool and Indian strain No. 1 by this method using inocula of 10 c.c. The results were satisfactory in that PR8 and Lee could be differentiated from the Λ prime Liverpool and the Indian strain: but the titres obtained were low—128 with the Λ prime strains and 256 with PR8 (Table II). This was perhaps due to the fact that the allantoic fluids used as antigens gave, in general, titres of about 256 only. It was possible to increase the titres of the allantoic fluids by further passage in eggs, but it was felt that this may result in antigenic deviations from the original isolation.

TABLE II.

Titration of immune sera against strain 1, PR8 and Liverpool.

Antisera			Antigens			
			Strain 1	PR8	Liverpool	Lee
Strain 1	128	< 8	128	< 8
PR8	32	256	32	< 8
Liverpool	128	32	128	< 8

The possibility of preparing hyper-immune sera by repeated injections was then considered, despite the likely danger of some loss of specificity in the process. Four roosters weighing between 4 lb. and 8 lb. and four rabbits of about 1,500 g. were taken and immunized with strain 1 antigen by different routes, viz. intravenous, intraperitoneal, one intravenous followed by intraperitoneal and one intraperitoneal followed by intravenous injections. The inhibitor content of rabbit sera was removed by treatment with crude cholera filtrate before use, while fowl sera were de-activated and used. The results are given in Tables III and IV. One of the rabbits died during the experiment, but no fowl was lost out of the 35 birds used so far for the study.

TABLE III.

Homologous antibody titres of sera after first, second and third inoculations with strain 1 in fowls and rabbits.

	Rooster				Rabbit		
	1 (IP)	2 (IV)	3 (IV&IP)	4 (IP&IV)	A (IP)	B (IV)	C (IP&IV)
1st immunization	256	128	128	32	32	128	64
2nd ,, ...	4096	1024	512	1024	128	> 4096	128
3rd ,, ...	> 4096	> 4096	> 4096	> 4096	> 1024	> 4096	1024

TABLE IV.

Antibody titres of sera from fowl No. 4 and rabbit C against seven strains.

Strains		Rooster 4			Rabbit C		
		1st	2nd	3rd	1st	2nd	3rd
Strain 1	...	32	1024	> 4096	64	128	1024
PR8	...	8	8	8	64	64	16
FM1	...	8	256	1024	< 8	< 8	128
Liverpool	...	32	1024	1024	64	256	256
Sweden	...	32	512	512	64	128	128
Lee	...	< 8	< 8	< 8	32	128	32
Crawley	...	8	16	16	32	64	32

It is clear from the results in Tables III and IV that the loss of specificity expected from hyper-immunization does not take place. Differences between homologous and other strains not evident after one injection are clearly brought out after three injections. Thus, serum I from fowl No. 4 shows antibody titres of 32 equally against strain 1. Liverpool and Sweden, serum II inhibits Sweden to 512, and strain I and Liverpool to 1024, while serum III gives a titre of >4096 against strain I without any further increase against the Liverpool and Sweden strains.

The route of immunization does not appear to influence appreciably the titres or specificity of the sera allowing for individual biological variations. Antisera were subsequently prepared in fowls by giving one intraperitoneal injection of 10 c.c. followed by one or two intravenous injections of 5 c.c. each of the bacteriologically sterile virus-infected allantoic fluid pool. Over 35 birds have so far been immunized. They weighed between 4 lb and 8 lb. and were made up of different breeds, viz. desi, white Leghorn, Rhode Island red and Black Minorca. They were all satisfactory, but the Rhode Island birds were found specially suitable for bleeding due to their prominent veins. Sera were collected by bleeding, between 10 c.c. and 15 c.c. at a time, from the wing veins ten days after the first and seven days after the second and third inoculations, respectively. They were ampouled and stored at -20°C . De-activation at 56°C . for 30 minutes was carried out on the day of the test.

Many studies on antigenic analysis have been carried out several antigens being tested against a few standard antisera. In this inquiry antisera have been prepared against each one of the strains, so that a detailed study could be made of individual strain variations. The experiments are still in progress.

Infected allantoic fluids were used as the antigens. In the case of the Indian strains care was taken to see that they had undergone only two or three passages since isolation with one exception, viz. strain 2 which was in the 5th passage. Ten-day-old eggs were inoculated with 0.1 c.c. of 10^{-2} dilution of amniotic or allantoic fluids, incubated for 48 hours at 35°C ., chilled at 0°C . for not less than two hours and the allantoic fluids harvested. Sterile fluids with suitable hæmagglutinating titres were pooled and titrated again. Experiments were carried out to find the suitability of merthiolate solution 1 in 10,000 as a preservative. It was found that merthiolate in this strength did not interfere with the hæmagglutinating properties of the viruses in the inhibition tests even when they were tested six months after such treatment. One antigen, strain 1, however, showed a very interesting reactivity to the reagent. Attention was drawn to this by the very high titres to which the merthiolated antigens of this strain were inhibited not only by homologous and heterologous fowl and rabbit sera, but also by non-specific inhibitors, from normal sera. The merthiolated antigen seems, to show an unusual susceptibility to inhibition without any apparent change in its hæmagglutinating titres. Further studies are in progress on the nature of this character. The antigen pools were diluted when necessary to give hæmagglutinating titres between 128 to 256 for routine work and stored at 0°C . for the duration of the experiments.

Fowl cells were used for all the tests. Red blood cells from several fowls were collected from the wing veins in Alsever's solution, washed four times in saline and packed at 3,000 r.p.m. for five minutes. 0.5 per cent

suspension was made from the packed cells in physiological saline and standardized by using a photo-electric colorimeter to give a constant reading every day. Cell counts were made of the suspensions and they were found to give consistently counts of about 30,000 per cubic mm. Fresh cells were used for the day's test, while the stock of cells in Alsever's solution was kept for one week. Preliminary tests were made with cells from about 20 birds using lipoid material, such as the Wassermann antigen. Only those birds whose cells were insensitive to lipoid agglutination were used as a source of cells. For all the critical experiments from which antigenic ratios have been calculated cells of the same bird have been used.

A stock solution of 8.5 per cent sodium chloride was prepared in bulk, filtered and kept. The quantities required for each day were prepared by diluting the stock solution 1 in 10. The dilution was used up the same day, any quantities remaining being rejected. All tests were carried out in hæmagglutinating tubes, 50 mm. long and 10 mm. internal diameter with lips. They were suspended from the top of the racks by means of the lips so that readings could be easily taken by passing a mirror under them. Plastic plates supplied by the World Influenza Centre were utilized for some of the experiments. The difficulty of thorough mixing of the reagents by shaking experienced with the plates, led to the use of these tubes for the major part of the work despite the heavy labour involved in washing the tubes.

Doubling dilutions of the sera were prepared in bulk commencing with one in eight and going up to 8,192. Unit volumes of 0.25 c.c. were transferred from the dilution tubes into those actually used for the test. The antigens were added subsequently each unit volume containing eight hæmagglutinating doses calculated from titration carried out on the same day. They were re-checked by the back titration carried out at the end of the test in which doubling dilutions to give 4, 2, 1 and $\frac{1}{2}$ hæmagglutinating doses of each antigen were treated with unit volumes of cells. The cells were added to the mixtures of serum and antigen generally between 15 and 30 minutes after the addition of the antigens. The tubes were left at room temperature (18°C. to 20°C.) and readings taken exactly after one hour using a mirror. The end-point has been taken as the dilution giving complete inhibition though records have been maintained about readings for partial as well as complete hæmagglutination. No attempt has been made to use interpolated readings as it was felt that this might introduce further variables. Readings were also taken after leaving the tubes overnight at room temperature. They showed marked differences from strain to strain apparently due to the varying degrees of elution of the virus.

Automatic pipettes were used for measurements of all volumes. The experiments have been repeated several times, e.g. sera obtained from fowls after repeated injections have been put up for inhibition titration against homologous and heterologous strains on three or more occasions. Antigenic ratios have, however, been calculated only from those experiments where several sera and their antigens have been tested on the same day.

RESULTS.

The fowl has been found to be very satisfactory for production of antisera against influenza-virus strains due to its low Chu inhibitor content, ease of immunization and bleeding, and ready availability. The presence

of Francis inhibitor in normal fowl sera does not interfere with hæmagglutination inhibition tests as unheated antigens are not affected. Antisera against Sweden and Liverpool strains were tested after de-activation as well as after treatment with crude cholera filtrate. Antigenic ratios worked out from the results were identical. A certain amount of loss of antibodies resulted from treatment with crude cholera filtrate in this experiment.

Contrary to expectations, hyper-immune sera have been found to give higher titres as well as more marked specificity

Preliminary tests on different days with various antisera show that the 17 antigens studied to-date fall under four broad groups : (i) Type A—PR8 and strain 5 ; (ii) type A'—FM1, Lepine, Sweden, Liverpool and strains 1, 2, 3, 7, 8 and 9, and (iii) type B—Lee, Paddington, Crawley and strains 4 and 6.

Comparative hæmagglutination-inhibition tests were put up within each of these three groups using a number of sera and antigens on the same day. The antigenic ratios were calculated from the results using the formula $r = \sqrt{r_1 \times r_2}$, where r_1 and r_2 are found by dividing the respective heterologous titres by the respective homologous titres. The reciprocals of the calculated ratios are given in Tables V and VI. A high ratio indicates pronounced dissimilarity, while a value of 1.0 indicates complete identity and intermediate values, above as 1.4 a fair amount of relationship. The Indian strains 1, 2, 3, 7, 8 and 9 are closely related to each other and to the Liverpool strain. Strain 2 seems to show a close relationship to Sweden as well as Liverpool, while strain 7 is apparently less closely related to the Sweden than the remaining strains. Strains 4 and 6 appear to be practically identical in mutual cross reaction as well as in their differences from Lee and Paddington strains. Though both are closely related to the Crawley, strain 4 is slightly more so than 6 which was isolated seven months later.

Antisera are being prepared against the remaining six strains and further studies are in progress.

TABLE V.

Reciprocals of antigenic ratios calculated from the results of the tests with hyper-immune fowl sera.

A prime antigens.

Strains			Sweden	Liverpool
Sweden	...		1	2
Liverpool	...		2	1
Strain 1	...		1.4	1
„ 2	...		1	1
„ 3	...		1.4	1
„ 7	...		2.8	1.4
„ 8	...		2	1
„ 9	...		1.4	1

TABLE VI.

B antigens.

Strains		Lee	Crawley	Paddington	Strain 4	Strain 6
Lee	...	1
Crawley	...	5·6	1
Paddington		8	11·3	1
Strain 4	...	11·3	1·4	5·6	1	...
Strain 6	...	16	2	5·6	1	1

4. Virus Research Centre, Poona.

The Virus Research Centre is maintained by the Indian Council of Medical Research with the co-operation of The Rockefeller Foundation and the Government of Bombay.

The primary purpose of the V.R.C. is to isolate and study Indian viruses which are transmitted by blood-sucking arthropods and which are of importance to man, especially in India.

Two strains of virus have been isolated, but have not yet been classified by means of appropriate immunological methods. It is, therefore, not possible to say whether they are new viruses or merely new strains of known viruses.

With the onset of the monsoon in June, an active research programme was started. The programme consisted of a systematic attempt to isolate viruses and it required the full attention of the entire research staff.

Virology.—The material selected for virus studies comprises : (1) Blood serum from persons ill with unexplained fever, especially children of from one to two years of age ; (2) blood from fledgling birds, particularly the common crow, the common mynah and the common weaver bird, and (3) blood-sucking arthropods, particularly mosquitoes and mites. A detailed list of the material tested for virus is given in the *Appendix* of this report (pp. 358-60).

The infant albino mouse is the most susceptible animal now available for the isolation and cultivation of arthropod-borne viruses. The chicken embryo, though highly susceptible to infection with such viruses, is less suitable for isolation studies because of the difficulty of recognizing the presence of a disease in this host. Material to be tested for virus is injected into the brain tissue of infant or adult mice or both. The animals are observed for symptoms of disease and if they sicken, studies are made to determine whether bacteria are responsible for the disease. If no bacteria are found by smear and culture, and if the disease is reproducible in mice upon serial passage, it is assumed that the disease obtained is caused by a virus. The identification of a virus with some previously known virus depends on immunological tests. If a known immune serum obtained from an animal immunized with a given reference virus neutralizes the newly isolated virus, while a known normal control serum does not do so, the identity of the new virus is indicated. It is then known that the new virus is either identical with or antigenically related to the reference virus.

The information now available concerning the two strains of virus which have been isolated is given below. Though only one of the virus strains is pathogenic for mature mice, the two strains may be related or identical, because the disease produced in infant mice is similar.

Koel virus.—In the course of the study of fledgling crows, six fledgling koels were found in crows' nests. This parasitic cuckoo (*Eudynamis scolopaceus*) lays its eggs in crows' nests. The six fledglings were bled and the blood tested for virus in the same manner as for crows. The koels were kept alive for study.

One of the fledgling koels died of paralysis ten days after it was collected and from the brain tissue an infectious agent isolated which is pathogenic for infant mice but not for mature mice. The koel from which this infective agent was isolated was collected on 12th June, 1953, and killed on 23rd June, 1953, when it became paralysed.

Infant mice inoculated intracerebrally with dilutions of infected brain tissue up to $10^{-5.5}$ develop paralysis in from four to six days and become prostrate and die in from 24 to 48 hours. The paralysis is of the spastic type. Infant mice inoculated subcutaneously with ten per cent suspensions of infected tissue do not develop any illness. The brains of paralyzed mice show an acute degeneration of neuroglial tissue and infiltration of polymorphonuclear cells. No elementary bodies or visible micro-organisms are found in brain smears stained with Giemsa stain. Cultures of infected brain tissue in brain-heart infusion show no growth. The infective agent is filterable through Berkfeld V grade candles and is resistant to glycerol.

In a single neutralization test, 100 infant mouse LD_{50} of the infective agent were neutralized by the blood serum of an adult man resident in Poona City. One human serum showed no protection and another partial protection against this amount of infective agent.

Crow virus.—A moribund fledgling crow, collected on 3rd July, 1953, died on the way to this Laboratory. The crow was autopsied and a ten per cent suspension of the spleen tissue was tested by intracerebral inoculation into six-to-eight-week old mice. One mouse was found paralysed on the 20th day after inoculation. Brain tissue from this mouse was negative for bacteria by smear and culture, and when inoculated into other mice by the intracerebral route reproduced a paralytic illness. The infective agent is now in the fifth passage and produces a paralytic disease in five to seven days in six-to-eight-week old mice inoculated intracerebrally but even in mice inoculated with a ten per cent brain-tissue suspension it does not cause a 100 per cent mortality. In infant mice it produces a uniformly fatal disease. Serological studies have not been done to determine whether people living in and around Poona have immunity to this disease.

Parasites in bird blood.—The examination of Giemsa-stained smears of bird blood has revealed the presence of four well-known parasites: hæmoproteus, plasmodium, leucocytozoon and filaria.

EPIDEMIOLOGY.

Virus Immunity Survey Continued.—During the period under review, two papers have been submitted (but not yet accepted) for publication,

which contain the results of neutralization tests done on 588 human sera, collected at 38 places in six states in Southern India in 1952, against 15 different neurotropic viruses. The neutralization tests were done in the Laboratory of The Rockefeller Foundation in New York by Dr. K. C. Smithburn and a preliminary report on them was included in last year's report.

The results obtained in this study are summarized below :—

Virus	Number of sera tested	Sera which contained neutralizing antibody	
		Number	Per cent
Yellow fever	588	0	0
Bunyamwera virus	195	0	0
Bwamba fever virus	190	0	0
Russian Spring-Summer encephalitis	588	8	1·3
West Nile virus... ..	351	123	35·0
Murray Valley encephalitis	242	21	8·7
Japanese B encephalitis	588	19	3·2
St. Louis encephalitis	211	6	2·8
Ilheus virus	206	3	1·4
Dengue type 1	176	72	40·4
Dengue type 2	188	38	20·2
Zika	196	33	16·8
Uganda S	197	21	10·7
Ntaya	247	23	9·3
Semliki Forest	192	3	1·6

Noteworthy is the high incidence of positives to West Nile and Dengue Type 1 and Dengue type 2, as well as the completely negative results with yellow fever and with the two African viruses, Bunyamwera and Bwamba fever.

Six of the eight positive result with Russian Spring-Summer encephalitis were obtained on sera collected in Kutiyana, a village near Porbander, Saurashtra. It is concluded that either this virus or else an antigenically closely related virus has been present at Kutiyana in recent years.

The positives with West Nile virus require further discussion because of the fact that this and four other viruses—Japanese B, Murray Valley, St. Louis and Ilheus—are known to be antigenically related. All of the sera listed as positive with each of these four viruses were also positive with West Nile virus, except one serum positive to Ilheus.

It is concluded that at least one virus belonging to this group is prevalent in India and that more than one virus may be present.

The positives with the three viruses—Zika, Uganda S and Ntaya, which are all of African origin—are interesting because the sera which were positive with each of these viruses were also, with very few exceptions, positive to one or more other viruses. A few such sera were positive to five different viruses,

The positive results were obtained on sera of apparently satisfactory quality in neutralization tests in which an adequate amount of virus was used (at least 50 LD₅₀ and usually 100 or more LD₅₀).

These three viruses have been shown not to be antigenically closely related to any of the other viruses used in the study. Thus, the results suggest that the three viruses—or antigenically related viruses—have been present in India. The somewhat puzzling phenomenon of 'multipositive' sera also, however, suggests that caution is indicated in the interpretation of the results.

At this stage it appears that the solution of the problem will have to await the isolation of viruses in India which are either identical with, or antigenically related to, the viruses in question.

Regarding Semliki Forest virus, the significance of the three positive results is not clear.

The only *new study* of retrospective immunity done in the period under review was very small.

When it was shown that immunity to West Nile virus was very widespread in India, it became of interest to find a place near Poona at which that virus might be searched for intensively. The place nearest Poona at which positive sera had been collected was Sirur, 40 miles north-east of Poona. Two of the ten sera collected there, from men aged 20 to 35 years, were positive, the donors of the two sera being aged 23 and 24 years, respectively. Thus, there was no proof of the recent presence of the virus.

To obtain more information about the presence or absence of the virus in recent years 11 children aged three to six years were bled in April 1953. Their sera were sent to Dr. Richard M. Taylor at Cairo, Egypt, for testing against West Nile virus at the Laboratory of the American Naval Medical Research Unit No. 3.

All the sera gave negative results, so it is concluded that Sirur is not a good place where special studies of West Nile virus should be made.

The search for a suitable place is still continuing and will be intensified when a reference strain of West Nile virus is available for use at the V.R.C.

The work done, has been largely preparatory, devoted to the training of subordinate personnel and to the development of working methods attuned to the special requirements of the V.R.C.

The technical work accomplished during the period may be summarized as follows :—

Laboratory colonies of *Culex fatigans* and *Aedes albopictus* have been established. A few imagoes of *Aedes aegypti* were collected, not without considerable difficulty, in Thana town, and brought to the Laboratory, but an attempt to establish a colony of *A. aegypti* was not successful.

Fairly extensive surveys of the non-anopheline mosquitoes have been made in and around Poona with three ends in view : (1) to ascertain what species are present, and common, in this area ; (2) to collect sizeable batches of the most common species to be tested for virus ; and (3) to establish catching stations to be used for the study of seasonal variations in the abundance of the various species found.

To-date a total of 42 species have been taken in the course of these studies, several of them not previously recorded in the vicinity of Poona.

A small portable mosquito trap, suitable for being suspended in a tree, baited with a small bird or mammal, has been devised and successfully used with a crow as bait.

A simple procedure for the easy recovery of arthropod ectoparasites from birds and mammals has been developed. (This procedure may be in use by others, but no published description of it has been found). The technique consists simply of wrapping a dead bird or mammal in a piece of cotton-lint, with the nap inside, and placing the specimen in the refrigerator over night at a temperature of about 4° C. When the specimen is removed from the refrigerator, sluggish but living ectoparasites are found entangled in the fibres of the nap. This procedure yields, in a few minutes of searching time, many times as many ectoparasites as can be found by direct search in the feathers of a dead bird.

The arthropod fauna of 88 birds' nests (crows 53, mynahs 16, and other birds 19) has been collected by means of a specially designed Berlese funnel. The only blood-sucking arthropods found so far have been *Macronyssid* mites, *Bdellonyssus*, *bacoti* being the commonest, and one species of *Culicoides*. A number of forms which do not feed on blood have been found in abundance in the nests, comprising: psocids, the larvæ and imagoes of several dipterans and coleopterans, as well as mites of the super-families Uropodina and Oribatei.

The ectoparasites of birds, including their fledglings, have been studied. As shown in the statistical summary, the total number of birds so studied was 247 with crows and mynahs comprising the majority of the specimens. Mallophaga have been found on practically every bird and *Macronyssid* mites on a few.

The detailed taxonomic study of this material has, of necessity, had to be deferred. Further information is given in the statistical summary which is attached.

ZOOLOGY.

The zoological activities of the V.R.C. are directed primarily toward small wild birds and mammals on the hypothesis that some of these warm-blooded vertebrates, together with the blood-sucking arthropods which parasitize them, may constitute a 'reservoir' of viruses which affect man and, perhaps, domesticated animals, such as horse, cattle, sheep and goat.

Major attention so far has been given to birds which are much easier to study and to collect than are the perhaps equally numerous small wild mammals. The latter will receive full attention in due course.

The work done to-date has involved mainly the identification of the birds which are present in Poona and vicinity; and the collections of selected species of birds, and their nests, for virus-isolation studies. Observations have already been started on the very important matter of seasonal presence and absence at Poona of those species which are migratory and are, therefore, present at Poona during only part of the year.

In the *Appendix* is given complete list of the wild birds and mammals collected, as well as of bird nests.

Comments on fledgling birds.—A newly-hatched bird is essentially an embryo. On the other hand, it is exposed to a variety of blood-sucking arthropods against which it is completely helpless. Profound changes take place in a fledgling during the three to four weeks it remains in the nest. When it leaves the nest it has almost acquired the characteristics of an adult.

The kaleidoscopic changes which take place during the first few weeks of a bird's life—changes which occur at no other period in its life—suggest that this short period should be a fruitful one in which to search for viruses which are transmitted by blood-sucking arthropods.

In Poona, as elsewhere, the majority of the wild birds have their breeding season during the monsoon. One of the first of the abundant birds to start nesting is the common crow (*Corvus splendens*) which builds its nests and starts to lay eggs even before the onset of the rains.

A number of reasons led to the selection of the common crow as the species of bird on which to make the first systematic attempt to isolate virus from fledglings, i.e. the abundance of crows ; the accessibility of their nests ; and the conveniently large size of the fledglings. As work progressed, it was found that the fledglings were hardy, in that they survived handling for several hours, and that the removal of one fledgling from a nest rarely led the parents to abandon a nest with other fledgling still in it.

As the peak of the crow-breeding season passed, attention was turned to the common mynah (*Acridotheres tristis*). The crow and the mynah are similar in that they are given to roosting in large numbers in a favourite tree. This should tend to make them prone to insect-borne diseases, such as bird malaria and filariasis, and possibly also to virus diseases which are transmitted by arthropods.

LABORATORY ANIMALS.

Swiss mice.—The most important laboratory animal for work with the general group of viruses in which the V.R.C. is interested is the albino mouse which is needed in large numbers for any extensive programme of activities.

On 6th December, 1952, a shipment of albino mice of the Swiss strain was received by air from the Laboratories of The Rockefeller Foundation in New York. This strain of mice is considered to be of greater than average susceptibility to neurotropic viruses in general. The shipment contained 65 young adult females, about half of which were pregnant on arrival and 35 young adult males. These were used to start the breeding colony at Poona, which has been kept in rooms which are not air conditioned. The mice have bred well, even during the hottest summer weather.

On Saturday, 26th September, 1953, the weekly mouse count showed a total of 4,778 mice, not including sucking mice. Of the total 1,759 mice were females in breeding.

The total number of mice used during the period was 3,608, of which 223 were females with litters of baby mice.

With a colony of mice as large as that of the V.R.C. thought must be given to matters which conserve floor space and cubic space. Racks have been designed and constructed which hold the maximum number of

mouse boxes and which are consistent with adequate ventilation and accessibility to the animal attendants who care for the mice.

The mice are kept in boxes made of galvanized iron sheets with a solid sheet for a bottom. The top is separate, it consists of galvanized iron woven wire, 1/4 inch mesh, set into a galvanized iron frame. The dimensions of the boxes and of the tops are kept within small tolerances so that any top will fit any box. This interchangeability of boxes and their tops saves an enormous amount of time, because it permits any box to be opened simply by lifting the top with one finger or a thumb.

The diet which is given to the mice is extremely simple : coarsely ground wheat —66 parts by weight, whole dried milk powder—33 parts ; and sodium chloride, B.P.—one part. This is fed either in the dry form, with water from a bottle ; or in the form of a cooked wet mash, without extra water.

Leghorn chickens.—The chicken, or fowl, embryo is a very useful laboratory animal for the study of certain viruses and the V.R.C. has a small flock of Leghorn chickens, which are kept in pens with woven wire floors, as well as sides and tops.

Rhesus monkeys.—They are almost indispensable for certain important studies of viruses. The V.R.C. has a small stock of *Macaca mulatta*, purchased from an animal dealer in Bombay.

Other laboratory animals.—It is contemplated that before long colonies of guinea-pigs hamsters and cotton rats will be established at the V.R.C.

APPENDIX.

VIROLOGY—Specimens tested for virus up to 30th September, 1953.

	Number		No. pools	No. arthro- pods
Human blood : (from patients with un- explained fever)	137	Arthropods :		
		Mosquitoes :		
Fledgling bird blood from :		<i>Culex fatigans</i>	... 48	1,664
Common crow ...	101	<i>Culex vishnui</i>	... 2	27
Common mynah ...	50	<i>Culex cornutus</i>	... 1	30
Weaver bird ...	15	<i>Culex biteniorrynchus</i>	... 4	146
House sparrow ...	8	<i>Aedes albopictus</i>	... 9	513
Four other species ...	10	<i>Aedes w-albus</i>	... 4	139
Koel ...	6	<i>Aedes aegypti</i>	... 1	3
		<i>Anopheles subpictus</i>	... 1	25
		<i>Uranotenia</i> spp.	10	266
Mature bird blood from :		Totals 80	2,813
Common crow ...	2	Mites :		
Common mynah ...	11	Macronyssidæ		
House sparrow ...	2	<i>Bdellonyssus</i> sp.	... 24	2,498
Red-vented bulbul	2	<i>Dermanyssus</i> sp.	... 1	21
Kite ...	1	<i>Steatonyssus</i> sp.	... 5	904
Total ...	208	Læleptidæ	... 5	319
Bird spleens of :		Uropodina	... 1	250
Common crow ...	22	Proctophyllodidæ	... 1	50
Common mynah ...	3	Total	... 37	4,042
House sparrow	3			
Seven other species	7	Ticks :		
Total ...	35	<i>Boophilus</i>	... 3	77
		<i>Hyalomma</i>	... 21	9
		<i>Argas</i>	... 2	64
		Totals	... 26	150
		Lice :		
		Mallophaga	... 1	152
		Anoplura	... 1	20
		Total	... 2	172
		Flies :		
		Culicoides	... 5	81
		Ceratopogonidæ (not culicoides)	... 1	9
		Phlebotomus	... 2	72
		Totals	... 8	162
		Arthropods : Grand total	133	7,339

Appendix—*contd.**Epidemiology—*

Number of human blood sera collected, for immunological examination young children, at Sirur, Poona district ...	11
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Entomology—

Collections of arthropods from :

(1) Bird nests, processed in Berlese funnels :

Common crow	...	53
Common mynah	...	16
Weaver bird	...	13
House sparrow	...	3
Other species	...	4
Total	...	89

(2) Birds, including fledglings :

Common crow	...	128
Common mynah	...	56
House sparrow	...	11
Weaver bird	...	9
Other species	...	43
Total	...	247

(3) Mammals, wild and domesticated :

Buffalo and oxen	...	11
Bandicoots, rats and mice	...	9
Bats	...	7
Sheep and goats	...	6
Shrews	...	4
Squirrels	...	1
Other species	...	7
Total	...	45

(4) Mosquito collections :

Outdoors, in foliage, or biting man	...	108
Outdoors, in traps with bird or mammal bait	...	18
Inside houses	...	61
Total	...	187

ZOOLOGY 1st May to 30th September, 1953

Birds collected, including fledglings:

	Number		
	Alive	Dead	Total
<i>Corvus splendens</i> —common crow	114	21	135
<i>Acridotheres tristis</i> —common mynah	62	2	64
<i>Ploceus philippinus</i> —common weaver bird...	19	0	19
<i>Passer domesticus</i> —house sparrow	14	0	14
<i>Eudynamis scolopaceus</i> —koel	6	0	6
<i>Molpastes cafer</i> —red-vented bulbul	2	3	5
<i>Riparia concolor</i> —dusky crag martin	5	0	5
Twenty other species	10	24	34
Totals	232	50	282

Appendix—*contd.*

Bird's nests collected :				Number
<i>Corvus splendens</i> —house crow	52
<i>Ploceus philippinus</i> —common weaver bird	16
<i>Acridotheres tristis</i> —common mynah	11
<i>Riparia concolor</i> —dusky crag martin	4
<i>Passer domesticus</i> —house sparrow	3
<i>Copsychus saularis</i> —magpie robin	1
<i>Corvus macrorhynchos</i> —jungle crow	1
Grand total				88

				Number		
Mammals collected :				Dead	Alive	Total
<i>Pipistrellus coromandra</i> —Indian pipistrelle						
(bat)	0	3	3
<i>Pteropus giganteus</i> —flying fox	3	0	3
<i>Funambulus palmarum</i> —three-striped palm						
squirrel	1	0	1
<i>Sunchus murinus</i> —house shrew	0	1	1
<i>Herpestes edwardsii</i> —common mongoose	1	0	1
<i>Rattus</i> sp.—rat	0	1	1
Grand total				5	5	10

5. Inquiry on the incidence, nature and types of coxsackie virus infections in Bombay City under Dr. D. D. Banker at the Seth G. S. Medical College, Bombay.

Collection of material for isolation of virus was started in April 1952. The material was collected and stored in deep-freeze cabinet at -20°C . A total collection of samples has been made from 87 'cases' so far. Of these, 47 have been patients suffering from paralytic poliomyelitis or disease resembling pleurodynia (most of them were admitted to Bai Jerbai Wadia Hospital for Children); 18 have been adult patients admitted to the K. E. M. Hospital; and 22 have been sewage samples processed and supplied by the Indian Council of Medical Research Polio Research Unit (*see Table*).

The sewage samples were found uniformly negative for presence of coxsackie virus.

During the last six months, faecal samples from 34 cases of poliomyelitis, pleurodynia, etc., were processed in the high-speed centrifuge at the Haffkine Institute and inoculated into newborn mice. Of these, eight were found positive for Coxsackie virus, all confirmed by the finding of characteristic histopathologic changes in the tissues of the paralysed mice. All these eight were cases of paralytic poliomyelitis (*see Table*). A total of 465 newborn mice belonging to 52 litters were inoculated in these experiments.

Those faecal samples which were positive for coxsackie virus were forwarded to the Indian Council of Medical Research Polio Unit at Grant Medical College, where they were inoculated intracerebrally into monkeys. Of the eight samples, two were found positive also for poliomyelitis virus, while the remaining were negative.

TABLE.

Isolation of viruses from material collected.

	Material from cases of paralytic poliomye- litis, pleuro- dynia, etc.	Sewage samples	Material from other cases	Total
Collection	47	22	18	87
Tested for presence of polio- myelitis or coxsackie viruses	34	22	...	56
Positive for coxsackie viruses	8*	8
Positive for poliomyelitis virus	4*	4

*In two of these cases, poliomyelitis and coxsackie viruses were present in the same samples.

Some of the processed faecal samples from paralytic poliomyelitis cases, negative for coxsackie virus were suitably pooled together and inoculated into monkeys. Two pools showed presence of poliomyelitis virus, (see table).

6. Rabies researches under the Director, Pasteur Institute of Southern India, Coonoor.

1. *To determine the value of five per cent Semple's vaccine in human treatment.*

Since 1946 an inquiry has been in progress at the Institute to determine (i) whether the five per cent Semple's vaccine manufactured by the Institute has any protective value under circumstances in which adequate control untreated groups have been observed, and (ii) whether there is any variation in the mortality rate as a result of treatment with five per cent Semple's vaccine compared with the one per cent vaccine used at the Institute during the years 1912-1924 for which similar data are available.

During the past six months 38,527 case cards of patients who received antirabic treatment during the year 1951 and 1952 have been analysed. The persons belonging to the different groups were traced and their welfare 6 to 24 months after treatment ascertained.

During the years 1946-1952 a total of 2,631 persons bitten by infective, proved or presumably rabid animals, have been observed.

The results among persons bitten by animals whose *infectivity has been proved* by one of the bitten persons or animals dying of rabies can be summarized as follows :—

Number of persons bitten	...	219
Number completely treated	...	147
Deaths from rabies among the completely treated		12 (8·2 per cent)
Number incompletely treated	...	13
Deaths from rabies among the incompletely treated		3 (23·1 per cent)
Number who died during treatment	...	10
Number untreated	...	49
Deaths from rabies among the untreated	...	21 (42·8 per cent)
Number untreated and those who died during treatment	...	59
Deaths from rabies among untreated including those who died during treatment	...	31 (52·5 per cent)

The data regarding persons bitten by (i) proved infective, (ii) proved rabid, and (iii) presumably rabid animals can be summarized as follows :—

Number of persons observed	...	2,631
Number completely treated	...	2,345
Deaths among completely treated	...	12 (0·51 per cent)
Number incompletely treated	...	55
Deaths among incompletely treated	...	3 (5·5 per cent)
Number who died during treatment	...	10
Number untreated	...	217
Deaths among untreated	...	21 (9·7 per cent)
Number untreated and those who died during treatment	...	227
Deaths among the untreated including those who died during treatment	...	31 (13·7 per cent)

It will be seen from the above figures that 8·2 per cent of persons bitten by proved infective animals develop the disease in spite of having a complete course of treatment, while the mortality rate is 42·8 per cent in the same group of persons when they are not treated. It would, therefore, appear that the treatment with the five per cent Semple's vaccine given by the Pasteur Institute, Coonoor, saves four out of every five persons who would otherwise have developed rabies and died.

If, on the other hand, we take into consideration all persons bitten by infective, proved or presumably rabid animals the mortality rate in the treated group is 0·51 per cent. This is about one-fifth of the mortality rate of 2·9 per cent reported by Cornwall for a similar group in his study. This would indicate that the results of treatment with five per cent Semple's vaccine are superior to those obtained with the one per cent vaccine used during the years 1912-1924.

2. *Studies on the histochemical changes in the central nervous system and other organs in rabies.*

The main purpose of the inquiry is to study the histochemical changes in the central nervous system and other organs in rabies and correlate the changes, if any, with the biochemical changes in the blood and C.S.F.

The necessary chemicals, stains, etc. have been collected and the various histochemical techniques standardized. The findings in normal animals are being studied.

INDUSTRIAL HEALTH

1. Industrial health research unit at the All-India Institute of Hygiene & Public Health, Calcutta.

1. PORPHYRIN EXCRETION IN COMMON TROPICAL DISEASES AND IN LEAD POISONING.

Excretion of excess porphyrin in urine has been recommended as an index of early lead poisoning. As porphyrin excretion is not limited to this disease but is found in diseases, such as hæmolytic jaundice, anæmia, cirrhosis of the liver, etc., and also due to the intake of some drugs, such as morphine, sulfonamide compounds, acetyl salicylic acid, etc., it is always necessary to eliminate these diseases before associating excess porphyrin excretion to damage due to lead poisoning.

India being a tropical country, with her inherent tropical diseases it was thought advisable to take up the question of utilizing excess porphyrin excretion as an index of lead poisoning only after such excretion has been studied in common tropical diseases, such as malaria, kala-azar, filaria, etc.

With this point in view, the urines of patients from the School of Tropical Medicine Hospitals were collected and the excess porphyrin excretion estimated semi-quantitatively in the following way :—

Ten c.c. of urine is treated with two drops of hydrogen peroxide (20 vol. per cent) and three drops of glacial-acetic acid. The porphyrin is then extracted from this with 2 c.c. of ethyl ether by shaking. This is then seen under ultra-violet light when the fluorescent colour of the ether layer shows if there is excess porphyrin in it. In the urine of normal persons the colour of the fluorescence lies between green to blue. If excess porphyrin is present, the intensity of colour may vary from pink to red according to the concentration of porphyrin in the urine.

Table I gives a summary of the results obtained by studying cases of a few tropical diseases :—

TABLE I.

Disease	Number of cases excreting excess porphyrin	Number of cases excreting porphyrin within normal limits	Total number of cases studied	Percentage of the positive cases
Filaria ...	15	79	94	16
Kala-azar ...	9	25	34	26
Malaria ...	8	22	30	27
Miscellaneous cases ...	<i>Nil</i>	12	12	<i>Nil</i>
Control ...	<i>Nil</i>	20	20	<i>Nil</i>

It will be seen from Table I that quite a good percentage of patients suffering from the common tropical diseases mentioned above excrete excessive amounts of porphyrin. The χ^2 test carried out on the values in Table I shows that the excess porphyrin excretion may be attributed to the diseases mentioned, the value being $\chi^2=10.85$.

Studies of excessive porphyrin excretion were also done on workers exposed to lead. The urines collected from these workers were examined both for excess porphyrin and lead content. Table II presents the results :

TABLE II.

Urine	Number of cases excreting excess porphyrin	Number of cases excreting porphyrin within normal limits	Total number of cases	Percentage of the positive cases
Number of cases containing lead above 0.1 mg./litre of urine 	5	...	5	100
Number of cases containing lead below 0.1 mg./litre of urine 	3	20	23	13

χ^2 test applied to Table II shows high significance in that higher lead concentration in urine leads to higher excretion of porphyrin (χ^2 11.25).

In an attempt to correlate the concentration of lead in urine with excessive excretion of porphyrin it has been found that all the lead workers having concentrations of lead above 0.1 mg./litre excreted excess porphyrin. Excessive porphyrin excretion in cases with lower levels of lead was much less.

Thus, the study confirms that excessive porphyrin excretion may be taken as one of the useful indices for determining lead absorption. As the method is simple and does not require very elaborate arrangements except a source of ultra-violet rays, such as a 'Woods Lamp' and a few test tubes, periodic examinations of urine of workers for excess porphyrin may be recommended as one of the precautionary measures against lead hazard. The cases thus found to be positive may then be subjected to determination of lead in blood and urine and other more elaborate examinations.

2. STUDY INTO EMPLOYEE ATTITUDE AND MORALE.

A morale survey in two engineering factories of Calcutta was conducted. This involved a depth interview of more than 1,000 workers on the basis of a standardized scale consisting of 40 attitudinal items. The primary object of the survey was to determine the variables that determine workers' morale and the exact nature and degree of influence of each of these factors.

The data from the first factory have been analysed. The analysis involved was rather extensive, e.g. it involved calculations of about 125 X^2 tests. Significant correlations between morale and three variables were found. Tables III, IV and V give the relation of morale to union, to production bonus and to expected income, the significance of the relation being determined by the X^2 test:—

TABLE III.
Relation of morale to union membership.

Group	High	Low	Total
Union group	41·3	58·7	100
Non-union group	71·2	28·8	100

$X^2=28\cdot11$ significant to the one per cent.

TABLE IV.
Relation of morale to production bonus.

Production bonus	High morale	Low morale	Total
Rs. 14 and less	45·9	54·1	100
Above Rs. 14	59·7	40·3	100

$X^2=3\cdot991$.

TABLE V.
Relation of morale to expected income.

Expected income	High morale	Low morale	Total
Rs. 180 and below	54·6	45·4	100
Above Rs. 180	42·1	57·9	100

$X^2=9\cdot383$.

The influence of these factors on morale has been further corroborated by controlling on influences of each other and of other factors that may be affecting morale at the same time.

The data pertaining to the interview of the second factory have not yet been fully collected. Analysis would be undertaken as soon as it is complete and results duly communicated.

3. SALT AND WATER METABOLISM.

The last experiment in the series 'Salt and water metabolism and its seasonal variation' has been completed in last summer on similar lines as in the past. In this particular experiment, the scope of the object was spread up in manifold ways. Apart from studying the salt and water balance with the previously adopted methods (determinations of alimentary

intake and urinary and faecal output) the following studies were also undertaken : (1) A follow-up of the water loss due to sensible and insensible perspiration, by weighing method, (2) calculations of loss of water along with respiration, (3) calculations of metabolic water production, and (4) studies of electric conductivity and salt content of sweat.

Seven human subjects obtained an unrestricted diet for a total period of twelve days. Whatever they took during the period was weighed and recorded individually. Chemical analysis for chloride and water was done on each of the representative foodstuffs. From these figures, the total daily intake of salt and water was calculated for each subject. On the output side, urine and faeces were weighed and aliquot samples analysed for water and chloride.

Of the total period, the subjects remained indoors and sedentary for the first five days but on the last five days they undertook a measured muscular exercise each day. The exercise consisted of walking at a speed of three to four miles per hour for one hour in the morning and one hour in the evening. The energy expenditure during walking was determined with the Douglas-bag method. Collection of expired air was done from 25th to 30th minute of the walk and was performed on five subjects. Table VI shows the oxygen consumption and energy expenditure per minute of the five subjects during the exercise :—

TABLE VI.
Energy expenses during exercise.

Subject	Age	B.S.A. m ²	Environmental temperature, °C	Relative hu- midity of environ- ment, per cent	Pulse rate/minute at 30th minute of walk	Oxygen consump- tion in liters per minute	Energy expendi- ture in cal. per minute	Energy expendi- ture in cal./min./ m ² B.S.A.
S.S.S. ...	39	1.57	33	71.0	100	0.653	3.265	2.080
A.R.M. ...	38	1.40	32	Not taken	130	0.861	4.305	3.075
A.S.G. ...	24	1.82	35	68.4	120	0.808	4.040	2.220
S.P.B. ...	32	1.69	33	81.0	110	0.750	3.750	2.219
P.N.S. ...	22	1.52	32	79.5	140	0.721	3.605	2.372

The average energy expenditure was 3.79 kcal./minute, equalling 2.39 kcal./minute per square meter of body-surface area. Thus, the average energy cost for the two hours' daily work was 455 kcal. By use of a value of 1 kcal./minute for a corresponding period of rest, it is found that the extra energy output due to walking was roughly 335 kcal. per man per day. Such an excess work metabolism may not seem to be very high but it must be remembered that the experiments were done during the hottest part of the year.

Table VII shows the net picture of salt and water balance for both the rest and work periods. The figures given in Table VII are all calculated

on the basis of chlorides expressed as sodium chloride. Therefore, small deviation may be expected in balancing the figures of output and intake, as part of the chlorides are excreted as NH_4Cl .

TABLE VII.

Salt and water balance of seven subjects (Indian male adults) during five sedentary and five exercise days in May 1953.

(Each figure is the average of five days).

SALT BALANCE EXPRESSED AS NaCl IN G.				WATER BALANCE IN G.		
Subject	Intake through food and drink	Output through urine and faeces	Difference	Intake through food and drink	Output through urine and faeces	Difference
A.S.G.						
Rest ...	18.09	20.06	-1.97	5,716	2,150	3,566
Exercise ...	17.02	16.94	+0.08	5,993	1,897	4,096
A.B.S.						
Rest ...	15.49	10.31	+5.17	5,654	1,399	4,255
Exercise ...	13.72	8.53	+5.20	5,360	1,660	3,700
S.P.B.						
Rest ...	18.38	19.99	-1.60	5,896	2,169	3,727
Exercise ...	17.08	15.67	+1.41	5,907	2,354	3,553
S.S.S.						
Rest ...	14.99	13.61	+1.38	5,310	1,434	3,876
Exercise ...	13.00	12.05	+0.95	5,706	1,647	4,059
A.R.M.						
Rest ...	13.19	15.83	-2.64	4,422	2,005	2,417
Exercise ...	14.40	14.84	-0.44	5,148	2,587	2,561
S.C.S.						
Rest ...	14.87	16.27	-1.41	5,976	2,181	3,795
Exercise ...	13.68	15.28	-1.60	5,968	2,628	3,340
P.N.S.						
Rest	16.45	18.22	-1.77	5,264	2,076	3,188
Exercise ...	16.40	18.98	-2.58	5,707	2,457	3,250
Overall average :						
Rest ...	15.92	16.33	-0.41	5,463	1,916	3,546
Exercise ...	15.04	14.61	+0.43	5,684	2,176	3,508

Calculations on data regarding water and salt losses through sweat are still under work and will be reported later on. It may, however, be mentioned that along with the chemical studies of the composition of sweat, an attempt was made to work out a simple field method for estimation of the electrolyte content of small quantities of sweat. Two methods were employed: (i) determination of the specific gravity of sweat with a drop method and (ii) determination of the electrical resistance of a small sweat volume. The former method was not found useful but the latter gave promising results. This is illustrated by the preliminary data summarized in Table VIII. The determinations of the electrical resistance were made with a Wheatstone-bridge apparatus working on A.C., so that the polarization was almost entirely eliminated. A special microcapillary tube with platinum electrodes was designed for the purpose and the absolute values of the electrical resistance are naturally dependent upon the characteristics of this tube.

TABLE VIII.

Relation between chloride content of sweat (expressed as NaCl) and its electrical resistance.

Number of observations	Average NaCl content, per cent	Average electrical resistance 10^{-6} ohms
1	0.053	0.204
5	0.134	0.157
11	0.197	0.133
4	0.247	0.115
3	0.360	0.072
1	0.480	0.070

4. THE ANIMAL EXPERIMENT ON TOXICITY OF MINERAL DUSTS.

The reactions of the body tissues to dusts have been studied by different methods by a large number of workers. It appears that the tissues of the different parts of the body react essentially in a similar manner to those foreign bodies.

The peritoneal cavity is a site where these reactions may be produced and studied conveniently. The peritoneal response is also of such a character that it may be used as a basis for the classification of the dusts from a physiological standpoint and of the potential harmfulness of the dust. In the present study, dusts of ten minerals commonly worked upon in India were introduced intraperitoneally.

A fixed dose of each dust was introduced into the peritoneal cavity of guinea-pigs. The animals were killed at intervals and the peritoneal reactions studied. The initial reaction is always the production of peritoneal nodules. Their subsequent behaviour may be classified as: (1) Proliferative, (2) inert, and (3) regressive or absorptive. Of the ten mineral dusts, quartz, mica-schist, Barakar sandstone and mica-pigmatite showed strongly proliferative reactions and, therefore, are potentially hazardous. The behaviour of the nodules produced by Muscovite schist, carbonaceous shale and Hosier-Guiness were anamalous and should be studied further.

Biotite quartz and chalcopyrite also showed a proliferative type of reactions despite their low free silica content. Dolomite with lead-zinc ore from Zowar and Rajputana showed regressive type of reactions and is presumably innocuous. It is suggested that these studies should be further extended to see how these nodules ultimately heal, as well as to clear up some of the anomalous reactions.

5. THE HEALTH SURVEY OF WORKERS IN A RUBBER FACTORY.

The survey as envisaged in the last year's proposals did not proceed according to plan. Only about 20 per cent of the total number of workers volunteered for the survey. Some of the collected data are summarized in Table IX :—

TABLE IX.

Ages, anthropometric data, arterial blood pressures and hæmatological data of a group of workers in a rubber factory.

	Mean	Median	Mode	Standard deviation
Age (years) ...	28·91	27·57	25–29	7·02
Height (inches) ...	64·49	64·00	64·00	2·37
Weight (pounds) ...	104·58	101·58	100–109	13·55
*Per capita income ...	36·06	27·43		24·32
† { Systolic B.P. (mm mercury)	105·2	103·69	100–104	9·99
Diastolic B.P. (mm mercury)	68·05	67·31	60–64	9·30
Pulse pressure (mm mercury)	37·23	35·75	30·34	9·15
Girth (inches) ...	25·75	25·82	25–29	3·21
Chest expansion (inches) ...	1·46	1·5	1·5	0·5
Hæmoglobin (g. per cent) ...	12·91	13·23	13·0–13·9	1·73
R.b.c. (million/c.mm.) ...	4·16	4·23	4·0–4·4	0·66

*Per capita income = total income of the family the number of persons in the family.

The approximate income of all persons contributing towards maintenance of the family were added up. There are persons having family at homes where they were largely dependent on the produce of the land. The approximate monthly income from the land was also taken into account.

†B.P. was taken sitting.

Serology (Kahn test).—

111 persons gave negative reaction (88·9 per cent)

12 persons gave positive reaction (9·5 per cent)

3 persons gave doubtful reaction (2·6 per cent)

2 sera were discarded.

Mass chest x-ray.—Of 391 minatures taken 11 people with suspicious pictures were asked to come for retakes for large size films. Only seven attended of whom five had definite tuberculous infiltrations.

6. THE STUDY OF EFFECTS OF JUTE DUSTS ON MONKEYS.

A group of experimental monkeys are being kept in a large jute mill near Calcutta. Some of the animals have been there for nearly a year. Another group of monkeys has been added to these during the last six months. All these animals are kept in the dusty atmosphere of the batching shed in which three-shift work is going on all over the 24 hours. The monkeys are, therefore, exposed to the jute dust in amounts of at least three times more than the average worker. The animals are weighed weekly and periodically x-ray films of their chests are being taken. It is proposed to maintain these animals for two or three years and then study the effects on various organs due to inhalation of jute dust.

7. A STUDY INTO THE INCIDENCE OF SERO-POSITIVE CASES AMONGST TRAINEE RATINGS.

More than 500 trainee ratings in a Government of India training ship have been examined for the presence or absence of syphilis by serological tests.

A very large proportion of the boys come from lower middle-class of Bengalee homes. Of 527 boys tested so far, 471 are Bengalees. Though their ages range from 15 to 26, about 92 per cent fall in the age group between 18 and 22. All of them were physically quite fit.

The serological reactions were classified in four groups : Negative, positive, doubtful and discard (broken tubes, excessive hæmolysis, etc.).

Results were as follows :—

	Per cent
Negative	97·4
Positive	1·7
Doubtful	0·9
Discards	<i>Nil</i>

8. CLINICAL ACTIVITY.

The Unit has also had to investigate and advise patients with suspected occupational diseases referred to us mainly by factory authorities, Chief Inspector of Factories, West Bengal, and occasionally by private practitioners.

Though lacking in adequate facilities for hospitalization and even for out-door examination, we have so far dealt with 25 patients as out-door cases and in addition hospitalized and investigated 11 patients in co-operation with the School of Tropical Medicine.

An Asbestos Cement Factory, where a survey of the environments was carried out by the Unit and reported along with last years reports, provided us with 14 patients. These were mainly workers who had been most heavily exposed to asbestos and cement dusts. Though the concentration of asbestos particles at breathing levels in the grinding and mixing mills had been found to be well within the maximum permissible limit, the patients were investigated mainly for the presence or otherwise of pulmonary asbestosis.

Four of these patients with somewhat abnormal radiological findings of the chests were hospitalized in the School of Tropical Medicine hospitals. One of these in-door patients and one of the out-door patients were proved to be tuberculous. None of the patients showed any signs of pulmonary asbestosis.

Seven workers from the manganese (pyrolusite) grinding mill of a battery factory were examined for the presence or absence of manganese poisoning at the instance of the Chief Inspector of Factories, West Bengal. Complete clinical and neurological examinations failed to show any signs of such poisoning in any of the workers.

Eleven patients with skin disease with possible connection to their occupation were also examined. Five of them were hospitalized in the School of Tropical Medicine for investigation. Two of the patients were found to have allergic dermatosis of occupational origin.

9. STUDIES ON RESPIRATORY FUNCTION.

Some data will be given below on the variability of lung volumes and maximal breathing capacity among various parts of the population. These investigations were taken up during the past year as part of our studies of physiological norms in India and their variations with such factors as age, sex, occupation, physical activity, climate and certain diseases of occupational and other origin.

Table X shows complete data for lung volumes in recumbent posture of fifteen men with sedentary occupations, in ages ranging from 19 to 45 years. The residual capacity of the lungs was determined with the aid of a closed-circuit nitrogen-elimination procedure which was worked out for the double purpose to make the studies possible with a limited equipment supply and at the same time bring down certain experimental errors to a minimum. The vital capacity and its subdivisions were determined with the same experimental set up.

TABLE X.

Lung volumes in recumbent body position.

Subjects—15 Indian men (living in Calcutta) with sedentary occupations.

Age range—18 years to 45 years ; average age 31 years.

The volumes are expressed as saturated with water vapour at body temperature, and at prevailing barometric pressure (BTPS).

Volume	Litres		Litres/m ²		Percentage of total capacity	
	Mean	Range	Mean	Range	Mean	Range
Inspiratory reserve capacity ...	2·10	1·18-2·90	1·28	0·83-1·77	44·9	29·5-60·0
Tidal volume ...	0·65	0·41-1·28	0·40	0·25-0·82	13·8	8·5-28·3
Expiratory reserve capacity ...	0·83	0·36-1·38	0·51	0·22-0·93	17·7	8·3-28·5
Vital capacity ...	3·57	2·84-4·52	2·18	1·69-2·79	76·4	71·0-83·8
Residual capacity	1·15	0·78-1·64	0·70	0·48-1·04	24·5	17·7-29·5
Functional residual capacity ...	1·98	1·42-2·95	1·22	0·85-1·97	42·2	31·1-55·8
Total capacity ...	4·68	3·74-6·01	2·87	2·38-3·73	100·0	...

Special mention may be given to those figures in Table X that express the relative size of the residual and functional residual capacities. It is seen that the residual capacity was on average 24·5 per cent and the functional residual capacity 42·2 per cent of the total capacity of the lungs. These figures do not differ significantly from comparable results in Europe and the U.S.A.

Table XI shows the age distribution of the vital capacity in standing posture of 81 men with sedentary work and 121 jute-mill workers :—

TABLE XI.

Vital capacity in standing posture of 81 men with sedentary occupations (Calcutta) and 121 male jute-mill workers (West Bengal).

Subjects	AGE IN YEARS		Number of subjects	AVERAGE VITAL CAPACITY (BTPS)	
	Average	Range		Litres	Litres/m ²
Middle-class men with sedentary occupations	18·5	17-19	11	3·95	2·50
	22·4	20-24	10	3·74	2·48
	28·1	25-29	10	3·70	2·22
	32·2	30-34	11	3·41	2·13
	36·7	35-39	12	3·51	2·13
	41·3	40-44	13	3·42	1·90
	47·1	45-49	10	3·33	1·98
	54·3	52-55	4	3·15	1·95
Male jute-mill workers	28·2	23-33	66	3·52	2·41
	43·4	38-51	55	3·43	2·33

It will be seen from Table XI that a considerable drop in the vital capacity occurs between the ages of about 25 and 40 years among the sedentary workers. Among the jute-mill workers, on the other hand, the figures are only insignificantly lower in the older group (average 43·4 years) as compared to the younger (average 28·2 years). A comparison of the jute-mill workers with the sedentary workers in the corresponding age groups revealed that the vital capacity per square meter of body-surface area was significantly larger among the former. This difference is probably connected with the different levels of physical activity of the two groups of workers. It may be mentioned that the averages for the vital capacity in the present report are generally higher than previous Indian data and approach those reported from Europe and the U.S.A. for subjects with comparable body-size.

Table XII shows observations on the variability of vital capacity with season, posture and method of determination.

TABLE XII.

Variability of vital capacity in Indian subjects under different conditions.

Variation with	Vital capacity at	Vital capacity litres (BTPS)	Number of subjects	Difference between (1) and (2) in litres	Analysis of significance of difference t p	
A. Posture	(1) Standing position	3·74	15	$0\cdot209 \pm 0\cdot049$	4·268	< 0·001
	(2) Recumbent position	3·53				
B. Season	(1) Standing position, comfortable winter season	3·73	13	$-0\cdot037 \pm 0\cdot037$	1·009	0·10–0·50
	(2) Standing position, hot pre-monsoon season	3·77				
C. Method determination	(1) Recumbent position, air breathing	3·53	15	$-0\cdot039 \pm 0\cdot095$	0·720	0·10–0·50
	(2) Recumbent position, O ₂ breathing	3·77				

It will be seen from Table XII that six per cent lower figures, on average, were obtained in recumbent posture as compared to standing. No significant differences, on the other hand, were obtained during the comfortable winter season and the hot pre-monsoon season. Table XII also illustrates that no significant differences in vital capacity were obtained by use of two different methods of determination, i.e. single maximal respirations during oxygen breathing in a closed-circuit system with CO₂ absorption and during air breathing.

Table XIII shows the maximal breathing capacity in standing posture of 33 men with sedentary occupations and 123 male jute-mill workers. The determinations were made by collecting the expiratory air in a large spirometer during 30 seconds of maximal hyperventilation. It may be mentioned that no significant difference was obtained between this method of sampling and the use of Douglas bags.

TABLE XIII.

Maximal breathing capacity in standing posture of 33 men with sedentary occupations (Calcutta) and 123 male jute-mill workers (West Bengal).

Subjects	AGE IN YEARS		Number of subjects	AVERAGE MAXIMAL BREATHING CAPACITY (BTPS)	
	Average	Range		Litres/minute	Litres/minute
Middle-class men with sedentary occupations	24·9	19–29	17	106·9	65·6
	35·6	30–45	16	94·4	55·2
Male jute-mill workers	28·2	23–33	67	95·4	65·0
	43·3	38–51	56	86·0	58·3

The results in the Table XIII indicate a decrease in maximal breathing capacity with increasing age. Further data are being collected to allow an accurate comparison between the sedentary workers and the jute-mill workers and a comparison with Europeans living in Calcutta.

10. POSTURAL CIRCULATORY ADJUSTMENTS DURING DIFFERENT SEASONS, INCLUDING A COMPARISON BETWEEN PEOPLE WORKING IN AIR-CONDITIONED AND NON-AIR-CONDITIONED ROOMS.

Failure of the blood circulation to adjust itself adequately to the hydrostatic stress in the upright body posture is an important cause of physiological fatigue in occupational work and otherwise. As it is to be expected that hot climate may add to the difficulties of the body to adjust itself to the upright posture, a study has been started on a group of subjects of the postural circulatory response during different seasons. Up to now, a simple test consisting of pulse-rate and blood-pressure determinations in recumbent and standing postures has been performed.

Table XIV shows climatic characteristics of the experimental room during the actual tests in the winter, pre-monsoon and monsoon seasons. Averages for body-weights are also given in the table, indicating that no major deviations in water balance occurred in spite of the quite large range of climatic variation.

TABLE XIV.

Averages for dry and wet-bulb thermometer readings in experimental room, and body-weights in the morning of eleven male subjects studied during three different seasons.

Season		Dry-bulb temperature, °F.	Wet-bulb temperature, °F.	Body-weight, (net), lb.
Winter	...	75·7	62·2	122·7
Pre-monsoon	...	91·4	77·6	122·8
Monsoon	...	88·2	80·7	123·4

Tables XV and XVI show the average results for the pulse rate and arterial blood pressure, respectively.

TABLE XV.

Pulse rate in recumbent and standing postures.

Average for eleven male subjects during three different seasons.

Season	PULSE RATE/MIN.										
	Recumbent.		Standing								
	16th min.	17th min.	1st min.	2nd min.	3rd min.	4th min.	5th min.	7th min.	8th min.	9th min.	10th min.
Winter	74·1	72·5	90·3	88·5	89·1	89·2	89·5	88·5	90·3	89·1	91·0
Pre-monsoon	72·5	72·5	97·5	94·2	92·5	90·6	90·9	90·5	91·5	90·8	91·3
Monsoon	75·9	76·1	103·9	100·7	99·0	97·5	98·5	97·9	98·2	98·9	99·1

TABLE XVI.

Arterial blood pressure in recumbent and standing postures.
Average for eleven male subjects during three different seasons.

Season	ARTERIAL BLOOD PRESSURE, MM. HG								
	Recumbent 18th minute			Standing 6th minute			Standing 11th minute		
	Systol	Dias- tol	Pulse pre- ssure	Systol	Dias- tol	Pulse pre- ssure	Systol	Dias- tol	Pulse pre- ssure
Winter ...	108·2	70·0	38·2	112·3	79·1	33·2	112·7	82·3	30·5
Pre-monsoon	102·7	63·2	39·5	104·1	79·1	25·0	105·0	80·9	24·1
Monsoon ...	103·6	61·4	42·3	105·9	74·1	31·8	108·6	79·5	29·1

It will be seen from Tables XV and XVI that the general reaction on transition from recumbent to standing posture consisted of a rise in pulse rate and a drop in pulse pressure due to a considerable increase in diastolic pressure. T-tests performed on individual differences between different seasons have so far revealed that there is better than 95 per cent odds for the following statements being true :—

(1) The pulse rate in standing posture was higher during the monsoon than in winter.

(2) The systolic and diastolic pressures in recumbent posture were higher in winter than during the other two seasons.

(3) The systolic pressure in standing posture was higher in winter than during the other two seasons.

(4) The diastolic pressure after five minutes of standing was higher in the winter than in the monsoon season.

(5) The pulse pressure after five and ten minutes of standing was higher in the winter than in the pre-monsoon season.

Experiments are being made to analyse these seasonal variations in postural adjustment but it is still too early to report any results.

During the pre-monsoon season, a comparison was made with the same test between subjects working in air-conditioned and non-airconditioned rooms. These experiments were carried out in the mornings and evenings, just before and after the working hours. No differences were found between the two groups of subjects which might be connected with the fact that the air-conditioned rooms did not differ very much in temperature from the non-air-conditioned rooms.

11. SEASONAL VARIATIONS OF BASAL METABOLIC RATE.

During the past year, a study was started of seasonal variations of basal metabolic rate. In this study, the bulk of material has still to be collected and it is, therefore, too early to present any detailed results. The same is true for the following projects :—

12. STUDIES OF THE STEADY-STATE RANGE DURING EXERCISE IN TROPICAL CLIMATE, IN RELATION TO THE WATER AND ELECTROLYTE BALANCE OF THE BODY.

AND

13. CAPACITY FOR EXHAUSTIVE WORK AND ITS SEASONAL VARIATIONS.

2. Inquiry into the problem of traffic accidents and accident-prone personnel amongst bus and tram drivers in Calcutta under Shri S. K. Bose at the University College of Science & Technology, Calcutta.

During the year under review the industrial workers of the Metal Box Company's factory in Calcutta have been examined. The plan of study was to administer the chosen battery of tests to 100 multi-accident and 50 accident-free persons, and to see how the test scores would correlate with their accident records. Complete records of 86 multi-accident persons have been available because some had left or were discharged when tests were being administered.

The following tests have been used in case of the Metal Box workers :—

- (1) Intelligence : Dearborn Formboard.
- (2) Reaction time : Vernier Chronoscope with light and sound stimuli.
- (3) Dotting ability : The apparatus devised at the Calcutta Psychological Laboratory was used.
- (4) Space discrimination ability : The material used for this purpose was devised at the Calcutta Laboratory.
- (5) Temperament : A specially prepared Personality Inventory was used.

The dotting apparatus, previously employed in connection with the testing of tram and bus drivers, has been further improved. In its present form it gives an objective record of the dots rightly or wrongly given on a roll of paper with an electro-magnetic writing lever.

For the space-discrimination test thin strips of aluminium of about one-and-a-half inch in length and one-fourth inch width were prepared. A small hole was punched at the exact centre of 50 such sheets. In fifty others the holes were punched slightly to the right or the left of the central point. The hundred sheets were then mixed and the subjects were required to sort but quickly those which had the holes exactly at the centre. The design of the test was based upon the principle of Drake.

Interview with the individual workers was held as usual and the supervisors' ratings were obtained.

The composite test scores have been correlated with the accident figures for the first one year of service and for the total period of service.

Inter-correlations between the tests were worked out.

			Dotting	Reaction time	Space dis.	Intelligence
Dotting	0·36	0·40	0·07
Reaction time	0·36	...	0·13	0·25
Space discrimination	0·40	0·13	...	0·14
Intelligence	0·13	0·05	0·09	0·30

Co-efficient of correlation between composite test score and accident figures was (i) for one year 0·31, and (ii) for total period 0·34.

3. Inquiry into effect of sewage treatment and excreta disposal methods on intestinal parasites under Dr. T. R. Bhaskaran at the All-India Institute of Hygiene & Public Health, Calcutta.

SYNOPSIS.

Sampling and analysis of sewage and sludge from three sewage-treatment plants were continued during the year. Samples were collected at periodic intervals for microscopic examination and chemical analysis. These samples were examined for the presence of helminths and amœbic cysts. Chemical analysis of the samples included determination of suspended solids, oxygen consumption by permanganate and B.O.D. During the year 216 samples were examined for parasites and chemical analysis was carried out on 189 samples.

Results obtained from the activated sludge plant are summarized in Table I :—

TABLE I.

Effect of activated sludge treatment on removal of parasites, B.O.D., and suspended solids.

Plant	Number of observations	Average number of ascaris eggs per litre of sample		Percentage removal of ascaris eggs	Number of observation	Suspended solids (ave.)		B.O.D. (ave.)		Percentage removal	
		Raw sewage	Final effluent			Raw sewage	Final effluent	Raw sewage	Final effluent	Suspended solids	B.O.D.
Bhatpara	83	838	58·6	93·0	45	477·8	66·6	323·5	48·9	85·5	83·4
Batanagar	62	59·3	0·9	97·5	41	205	19·7	120·5	9·5	88·0	91·4

The data presented above show that activated-sludge treatment removes more than 90 per cent of the eggs of helminths from the sewage. In the Batanagar plant which is well operated, the removal of parasites is better than in the Bhatpara plant. Few parasites escape in the effluent and if the effluent is, however, to be discharged in a water source used for drinking purposes, it may be necessary to disinfect the effluent before it is discharged.

Observations on septic-tank-cum-trickling-filter treatment have been completed during the year. The concentration of parasites in the sewage at different stages of treatment in this plant is presented in Table II :—

TABLE II.

Concentration of parasites at different stages of treatment in septic-tank-cum-trickling-filter plant (Jute Mill—Belur).

Sample	Number of observations	AVERAGE NUMBER OF HELMINTHIC OVA PER LITRE OF SEWAGE SAMPLES	
		Ascaris	Hookworm
Septic-tank effluent ...	61	32·3	30·0
Effluent from trickling-filter treatment ...	61	8·0	13·3
Trickling-filter effluent after disinfection with bleaching powder ...	61	1·1	3·5

It may be observed that septic-tank effluent has less concentration of helminths than sewage treated by plain sedimentation (*vide* last year's report).

The efficiency of removal of parasites by the different treatments in this plant is summarized in Table III :—

TABLE III.

*Effect of septic-tank-cum-trickling-filter treatment of sewage.
(Jute Mill—Belur)*

Treatment	PERCENTAGE OF PARASITE REMOVAL				PERCENTAGE OF B.O.D. REMOVAL	
	Ascaris		Hookworm		Mean	Standard deviation
	Mean	Standard deviation	Mean	Standard deviation		
Trickling-filter ...	83	2	72·1	24·7	40·8	22
Chlorination with bleaching powder	98	4	92·2	17·5	66·3	17·1

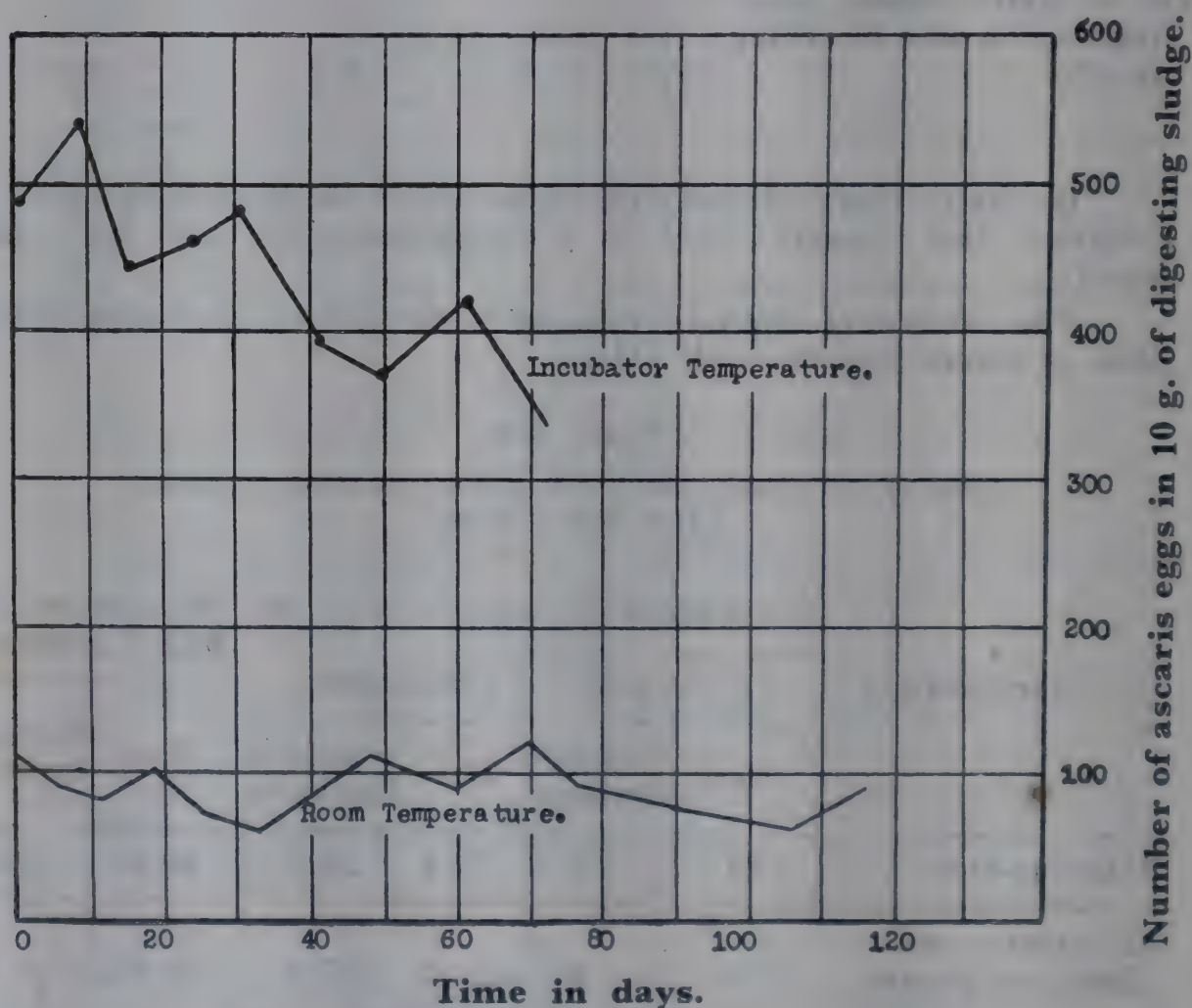
The results show that trickling-filter treatment removes over 70 per cent of the parasites from the septic-tank effluent. The chemical treatment of the filtered effluent with bleaching powder is very effective in

destroying the parasites that escape in the trickling-filter effluent. From the data so far collected it would appear that septic-tank-cum-trickling-filter treatment as is generally practised in the industrial establishments near Calcutta is as effective as activated-sludge treatment from the point of view of removal of helminths from the sewage.

The laboratory experiments on the effect of digestion of sludge under different conditions on parasites have also been carried out during this period. Sludge samples collected from the Bhatpara plant were used for this experiment. One hundred grammes of sludge were taken in glass-dishes and kept covered for varying periods in the open and inside an incubator maintained at 98°F. Evaporation losses from these dishes were made up by adding necessary amount of water to the dishes from time to time. The dishes were sampled periodically and the sludge was examined for the presence of parasites as well as to determine progress of the digestion process. The data obtained from these experiments are presented in the Figure :—

FIGURE.

Effect of sludge digestion on eggs of ascaris.



The ova isolated from the digesting sludge were also tested for their viability. It was found that a large percentage of the ascaris eggs (over 60 per cent) surviving digestion up to 40 days were found to be viable.

The results also showed that at the higher temperature of digestion there was significant destruction of parasites after about 25 days. More data are necessary before definite conclusions can be drawn on the effect of sludge digestion on parasites.

4. Inquiry into treatment and hygienic disposal of lac wastes under Dr. T. R. Bhaskaran at the All-India Institute of Hygiene & Public Health, Calcutta.

Pilot plant studies on treatment of wastes by trickling-filter were completed during this period. The results of the trickling-filter operation during the period are summarized in Table I.

TABLE I.

**Performance of trickling-filter for treatment of lac wastes.*

(Median value of 21 observations spread over a period of one year.)

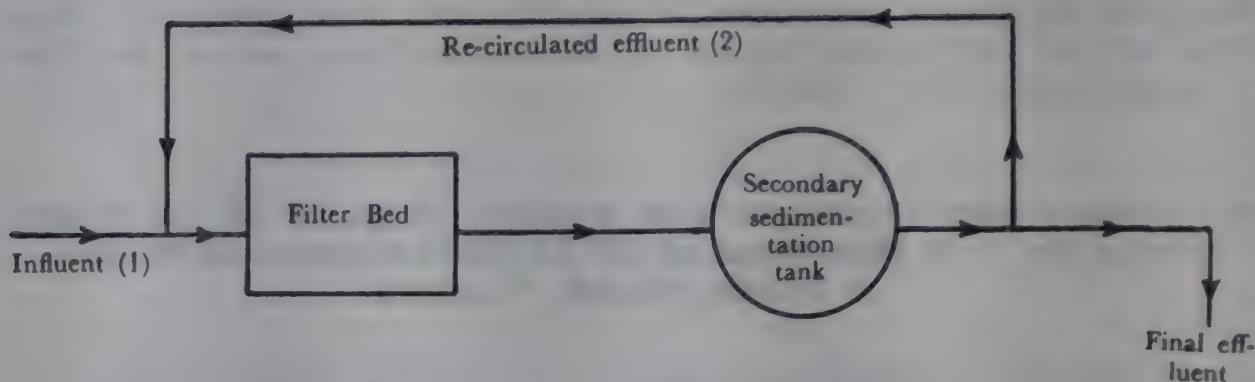
Item	Raw	Sedimented	Filtered	Overall efficiency as percentage removal
Suspended solids p.p.m. ...	11030	390	210	..
Per cent removal	96·8	42·5	98·3
Five-day B.O.D. p.p.m. ...	6500	700	150	...
Per cent removal	89·0	75·0	97·4
Colour p.p.m. ...	2000	500	150	...
Per cent removal	72·5	66·6	89·2
pH ...	5·10	6·65	7·20	...

*Filter loadings varied from 1,600 to 3,200 lb. of five-day B.O.D. per acre foot during the period of observation.

Results show that lac wastes are amenable to biological treatment in the trickling-filter. By this treatment about 98 per cent of the organic matter and about 90 per cent of the colour are removed. The effluent obtained by this treatment has a pH of 7·2 and it can be re-used for washing stick lac in its preliminary stages. Trickling-filter is suitable for treatment of wastes from large size factories where there are facilities for construction and operation of this type of plant.

Experiments on treatment of waste by biofiltration are now in progress. In this method the effluent from the filter is re-circulated. The flow of the waste with re-circulation is illustrated in the following flow diagram :—

FIG. 1

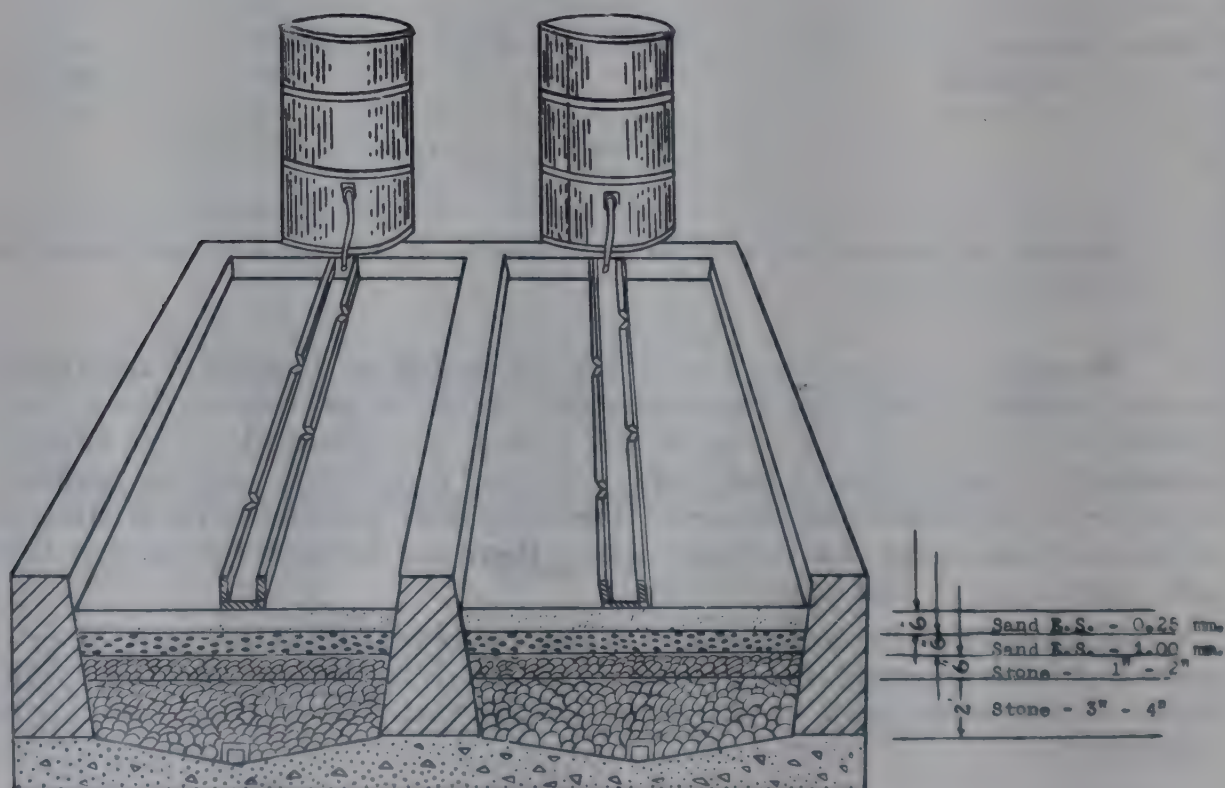


The results obtained so far indicate that it is possible to further reduce both B.O.D. and colour from the effluent by re-circulation.

The laboratory-scale experiments on ponding have also been completed during this period. Ponding of wastes for a period of about 20 days may be practised in areas where facilities for ponding are available. Ponding of the raw wastes for this period removes 80 per cent of the B.O.D. and 97 per cent of suspended solids from the waste. For factories producing about 5,000 gallons of wastes per day an area of nearly 5,000 sq. ft. is required for ponding.

Work on intermittent sand-filtration of the waste was also carried out during the period. An experimental sand-filter was constructed in one of the factories in Calcutta at a cost about Rs. 300. The pilot plant consisted of two filter units measuring $13' \times 3\frac{1}{2}' \times 3'$ each with concrete sides and bottom having a slope of one inch to drain away the effluent. One-and-a-half inch diameter G.I. pipe with a number of holes was placed at the bottom to drain away the filtered wastes from the unit. The filter media consisted of overburnt bricks and sand as shown in the figure. Lime-treated and settled waste was distributed over the filter bed with the help of a wooden channel kept at a slope of 2 inches and having overflow slots to permit uniform distribution of the liquid on the filter.

FIG. 2



Intermittent sand-filter — Pilot Plant.

In the present experiments the filter was dosed at the rate of 50,000 gallons per acre per day corresponding to a loading of about 310 lb. of five-day B.O.D. per acre foot.

Fifty gallons of settled wastes were dosed on the filter each time and this was filtered within a period of three to four hours. The filter was allowed to rest till next morning. The operation was continued till there was appreciable decrease in the rate of filtration due to choking of the bed. At this stage the operation was stopped, the surface mat of sludge was removed and the filter raked to clean the sand bed.

After the system had stabilized, representative samples of the wastes, before and after filtration, were analysed for different forms of solids, B.O.D., colour and pH. The results of operation of the filter for a period of four months are summarized in Table II :—

TABLE II.

Performance of intermittent sand-filter for treatment of lac wastes.

(Median values of observations spread over a period of three months).

Item	Influent sample I	After lime treatment and settling sample II	After filtration sample III	Over all efficiency (as per cent removed)
Suspended solids (p.p.m.) ...	10,400	4,430 (61·1)	60 (98·6)	99·4
Five-day 20°C. B.O.D. (p.p.m.)	7,500	1,800 (76·6)	85 (95·0)	98·9
Colour (p.p.m.) ...	2,500	1,200 (54·6)	45 (96·7)	98·5
Average pH ...	5·0	6·5	7·1	...

The results show that this treatment produces an effluent in which colour and B.O.D. are reduced to the extent of 99 per cent. The effluent obtained by this treatment can be used to augment the industrial water-supply in this region. Intermittent sand-filter plants are easy to operate and will be popular even amongst small-scale factories.

Further work to standardize conditions for the design and operation of the biofiltration and intermittent sand-filter plants for the treatment of wastes is in progress.

PHARMACOLOGY

1. Indigenous drugs inquiry under Dr. G. Werner at the School of Tropical Medicine, Calcutta.

A. SYNOPSIS OF THE WORK DONE AND RESULTS ACHIEVED.

I. (a) *Rauwolfia serpentina*.—The sympathicolytic action of total purified alkaloids of *Rauwolfia serpentina* of the individual pure alkaloids ajmaline, serpentine and rauwolfinine was found to be qualitatively similar to the corresponding activity of ergot alkaloids (of the competitive type) ; the antagonism concerns certain excitatory effects of adrenaline only and suppresses effects of sympathetic nerve stimulation to a similar extent as the respective action of adrenaline. A striking point of difference with known sympathicolytic drugs is that rauwolfia alkaloids do not cause reversal of the adrenaline-pressure effect.

Pressor as well as depressor vasomotor reflexes elicited by pressure changes within the carotid sinus or by electric stimulation of afferent nerves (carotid sinus nerves, sensory nerves or afferent vagus nerve) are blocked by R-alkaloids. This effect is independent of the peripheral sympathicolytic action of *Rauwolfia serpentina* : the relative potency of the various pure alkaloids for peripheral adrenergic blockade and central vasomotor depressant activity varies independently of each other ; on chromatographic separation of crude extracts over aluminium oxide, fractions were obtained with either predominant sympathicolytic action or with predominant suppressing effect on vasomotor reflexes. Vasomotor and respiratory reflexes elicited by stimulation of the chemo-receptors of the carotid sinus are not suppressed by R-alkaloids in a specific way.

Strongest support for the considerable contribution of a central effect to the overall hypotensive activity of *Rauwolfia serpentina* is obtained from experiments with intracisternal application of R-alkaloids (in anæsthetized and non-anæsthetized monkeys) : fall of blood pressure and inhibition of carotid sinus reflexes is obtained following the intracisternal injection of as little as a twentieth of the dose required to produce the same effect on intravenous injection. In the case of total purified alkaloids as well as of some individual pure alkaloids (ajmaline, rauwolfinine, serpentinine), the central effect on the vasomotor control was not found to be associated with a concomitant sedative or hypnotic action. The total purified alkaloids and the individual alkaloids mentioned do not even show any action on the behaviour of animals in 'sham rage' following decortication or in di-encephalic animals although such animals are rendered hypersensitive for drugs with sedative action as a consequence of cortical ablation (Dasgupta, Mukherjee and Werner, 1953).

Intracranial application of *Rauwolfia serpentina* alkaloids in decerebrate animals (mid-collicular level) does not give rise to any alteration of the mean arterial pressure nor do R-alkaloids suppress the characteristic pressure response elicited in such animals by transient artificial increase of the intracranial pressure. As an example for another drug interfering with the central regulation of vasomotor control, ergotamine was used in a comparative study : Ergotamine was found to lower the arterial pressure and to abolish the pressure response following rise of intracranial pressure,

in such decerebrate preparations. These findings indicate that the central effects of R-alkaloids of ergotamine differ from each other in respect to their localization, the structures involved in the response to ergotamine not being affected by R-alkaloids. The problem of the central localization of the effect of *Rauwolfia serpentina* on the vasomotor control is at present under further investigation, using the blood-pressure response following electric stimulation of circumscribed areas of the brain stem as test.

For the bio-assay of rauwolfia extract, the idea was further pursued to compare the hypotensive effect of R-extracts with the corresponding activity of known amounts of hypotensive drugs in animals with artificially raised blood pressure.

I. (b) It was found that of all the hypertensive agents so far used, viz. pituitrin, veritol, ephedrine, adrenaline, argyreia and hypertensin (prepared in this Laboratory), the last named principle was found most suitable for providing an initial high blood pressure. Histamine, aminophyllin and priscol were used as standards for comparison with the hypotensive activity of *Rauwolfia serpentina* extracts in such artificially hypertensive animals and it was found that 'priseol' was giving most constant and reproducible results. It appears that different rauwolfia extracts are best compared with priscol as standard for hypotensive activity in animals with artificial hypertension following administration of hypertension. This assay procedure refers to the overall hypotensive activity; if special properties of extracts (such as sympathicolytic activity or vasomotor reflex blocking action) should be determined, adrenaline antagonism or suppression of carotid sinus reflexes can be utilized as test; conventional statistical methods can be used to evaluate the data. By means of such assay procedures, it was seen that crude extracts are up to ten times more active in respect to hypotensive and sympathicolytic activity than one could expect on the basis of their alkaloid content (taking the known activity of pure alkaloids as standard of reference).

II. *Argyreia speciosa*—sweet (*Syn.* *Lettsonia nervosa*). *Roxb.*—An aqueous concentrate of the active constituent of *Argyreia speciosa* was tried as a hypertensive agent for the artificial increase of B.P. during the course of study of the biological assay of *Rauwolfia serpentina* extracts. Though the principle was found suitable in maintaining the rise of B.P., it was, however, abandoned as its mode of action has not yet been determined.

III. *Damia extensa*.—Studies on the keeping quality of the potency of the extract and the purified active principle used as an injectible revealed that, whereas the potency of the alcoholic extract of the drug remained unchanged even after a year, the potency of the purified principle started to diminish in appreciable amount after the lapse of about eight months. Various attempts are being made to stabilize the purified active principles and it is still premature to comment anything on their results.

On investigating the oxytocic action of *damia* and that of ergometrin it was noticed that they compared favourably. *damia* causes to start contractions of the uterus muscle at the fundus which then travels towards the cervix. Combination of oestrogenic hormones with *damia* appeared to be particularly effective in causing a strong hypermotility of predominantly the fundus of the uterus. Clinical tests to evaluate the effectiveness of the drug in parturition are in progress.

2. Indigenous drugs inquiry under Bt. Col. R. N. Chopra at the Drug Research Laboratory, Jammu.

A. INDIGENOUS DRUGS.

Our previous work has shown that there are more than fifty important essential oil-bearing plants growing in North-Western Himalayan region. A lot of chemical work in connection with these has already been done. It was, therefore, thought that these be screened with regard to their pharmacological properties. Five of these essential oils were investigated during this year.

Pharmalcalogical investigations of essential oils of Xanthoxylum alatum, Angelica archangelica, Alpinia galanga and Pistacia ingegrima.

1. *Xanthoxylum alatum* (vernacular—Timbroo).—This is an ever-green shrub which grows along the foot of Himalayas, from the Indus east wards to Bhutan and up to the Khasia Hills, at altitudes of 2,000 ft. above sea-level. The fruit has a sweetish, somewhat bitter taste. The drug is extensively used by Hakims and Vaidyas as carminative, stomachic and anthelmintic. The fruit contains 3·7 per cent of an essential oil, which has a lemon-yellow colour and a peculiar odour like that of water fennel. It is the demethyl ether of plora-octophenone $C_{10}H_{12}O_4$. Its specific gravity is 0·8653 at 15°C., refractive index 1·48134 at 20°C., acid value 0·9 and ester value 10·3.

2. *Angelica archangelica* (vernacular—Chura).—It is a large perennial erect herb, assuming the size of a small tree, and commonly occurs near the water channels at altitudes from 7,300 ft. to 8,000 ft., especially in Gulmarg and Gurez areas in Kashmir. It grows all over northern Europe and is also cultivated. The root and seeds have been used as an aromatic, diaphoretic, diuretic and externally as counter-irritant. Both root and fruit contain an essential oil, which is an almost a colourless liquid with a balsamic odour and becoming yellowish to brown in colour on exposure to air and light. The oil contains phallandrene and other terpenes, together with a sesquiterpene and esters of methyl (Valeric) and hydroxypenta decyclic acids. Its specific gravity is 0·859 at 15°C., refractive index 1·477 at 20°C., acid value 0·8 and ester value 13.

3. *Alpinia galanga* (vernacular—Kolanjan).—It is a small herb, which grows throughout India and is often cultivated in Ceylon and Malaya Islands. The rhizome has a sharp odour and a fairly good taste, and is used by Hakims and Vaidyas as stomachic, carminative, diuretic and expectorant. The roots contain three different compounds: camphenide, galangin, alpinin, and an essential oil to the extent of one per cent. The specific gravity of essential oil is 0·9847 at 15°C., refractive index 1·6163 at 20°C., and it contains 48 per cent methyl cinamate, 20 to 30 per cent cineol, camphor and d-pinene.

4. *Pistacia ingegrima* (vernacular—Kakar singi).—It is a tree nearly glabrous, which grows in outer ranges of North-Western Himalayas at altitudes from 1,500 ft. to 8,000 ft. above sea-level. The gall is bitter, hot and acrid. It is used by Hakims and Vaidyas as an expectorant in chronic bronchitis and asthma and pthisis. The galls contain 1·2 per cent of essential oil, a crystalline hydrocarbon 3·4 per cent, tannin substances 60 per

cent and gum mastric 5.0 per cent. The essential oil has a pale greenish-yellow colour with turpentine-like odour. Its specific gravity is 0.8885 at 15°C., and refractive index 1.4735.

All these four essential oils possess similar pharmacological activity and are, therefore, being dealt with collectively, minor differences, where they exist being mentioned.

Action on protozoa and bacteria.—The effects of the above four essential oils in different concentrations were studied on *Paramœcia caudatum*. In concentrations of 1 in 200 to 1 in 1,000, the organisms are killed in a few seconds. In concentrations of 1 in 2,000 to 1 in 10,000 the ciliates die in about five minutes. Dilutions over 1 in 20,000 have only slight effect. The essential oil from *Xanthoxylum alatum* in a dilution of 1 in 40,000 kills 50 per cent of ciliates in ten minutes.

The bactericidal properties of these oils were tested on *Staph. aureus*, *E. coli*, *S. typhosum* and *C. inaba* and *Sh. flexner*. The oils inhibit the growth of these organisms only up to a dilution of 1 in 500. The above studies show that these oils have only weak antiseptic properties.

Local action.—The essential oils of *Xanthoxylum alatum* and *Alpina galanga* have a marked irritant action on the skin and mucous membrane of rabbits. They also produce inflammatory reaction when injected subcutaneously and intramuscularly. The oils of *Angelica archangelica* and *Pistacia integrima* are only slightly irritant.

Action on cardiovascular system.—

(a) *Blood pressure.*—The action of the essential oils was studied on blood pressure of dogs anesthetized with phenobarbitone. Graded doses of the different oils, 0.01 c.c./kg. to 0.05 c.c./kg., given intravenously produced a fall in blood pressure, with subsequent rise but not to the original level. The heart rate is slowed and amplitude increases. In atropinized and dogs with vagi cut, similar fall in blood pressure was observed.

(b) *Blood vessels.*—The fall in arterial blood pressure was always followed by a decrease in intestinal volume. The action of the oils on the blood vessels of perfused hind leg of dogs showed vasoconstriction.

(c) *Heart.*—The myocardiographic experiments in anesthetized dogs showed diminution of amplitude of contractions of both auricles and ventricles, with doses, such as 0.02 c.c./kg. to 0.05 c.c./kg. The rate of contractions of auricles and ventricles is decreased. Larger doses produce irregularities. The isolated heart of the rabbit perfused with dilutions of oils ranging from 1 in 10,000 to 1 in 20,000 showed depression. The amplitude diminished and rate slows down. The heart recovers gradually, when drug solution is stopped. The dilutions over 1 in 50,000 have slight effect. The oils lower the blood pressure by directly acting on the heart muscle. This effect is not abolished even after the section of vagi. They have a vasoconstrictor effect on the blood vessels.

Action on gastro-intestinal tract.—The action of essential oils on the movements of small intestines was studied in dogs anesthetized with phenobarbitone. The essential oils in doses from 0.02 c.c./kg. to 0.05 c.c./kg. given intravenously produced slight increase in tone followed by subsequent relaxation. The isolated intestines of rabbit in Dale's bath showed relaxation in normal and those in which spasm was produced.

Action on respiratory system.—The effects of the essential oils were studied on the respiration of dogs anæsthetized with phenobarbitone. With graded doses of oil from 0.01 c.c./kg. to 0.05 c.c./kg., there is immediate depression of the respiration, followed by stimulation. Larger doses stop the respiration.

Action on the central nervous system.—The action of the above essential oils on the central nervous system is under study and will be reported subsequently.

Conclusions.—These essential oils appear to have weak antiseptic properties. They seem to share the carminative property of other essential oils, and have an anti-spasmodic action, inhibiting the excessive peristaltic movements of the intestines. The effects on the cardiovascular and respiratory systems are not marked and, therefore, not of much importance from therapeutic point of view.

Piper betel (vernacular—Pan).—Detailed investigations were taken up because of its widespread use by the people. It is a perennial creeper which is originally a native of Java but is now being cultivated in the hotter and damper parts of India and Ceylon. The plant is widely distributed under cultivation in Uttar Pradesh, Bengal, Central Provinces and Madras and a number of varieties are grown. The betel leaf according to Susruta is aromatic, carminative, stimulant and astringent. It sweetens the breath, improves the voice and removes all foulness from the mouth. It is also said to be useful in diseases supposed to be caused by deranged phlegm and its juice is much used as an adjunct to pills administered in these cases. The leaves contain starch, sugars, tannions, diastases (0.8 to 1.8 per cent) and essential oil to the extent of 0.2 to 1.0 per cent. The oil is a light-yellow liquid of aromatic odour and sharp burning taste. Its specific gravity is 0.958 to 1.057 at 15°C. The oil contains phenol (chavibetol an isomenids of eugenol), chavicol, cadinene and various sesquitenpenis. For our investigation we used betel leaves grown in Ahmadabad side which contained 0.41 per cent of essential oil. The pharmacological findings are recorded below :—

Action on bacteria.—The bactericidal effect of the oil was tested. 24 hours broth cultures of *Staph. aureus*, *B. typhosum*, *Cholera inaba*, *B. coli*, *Sh. flexner* and *Para A* 0.5 c.c. of various concentrations of the oil were added to 9.5 c.c. of the nutrient broth pH 7.2 which was inoculated with 5-mm. loopful of the culture, and incubated at 37°C. for 24 hours. The oil inhibits the growth of cholera vibrios in a dilution of 1 in 4,000, and that of *B. typhosum* and *Sh. flexner* in a dilution of 1 in 3,000. The growth of *B. coli*, *Para A* and *Staph. aureus*, was inhibited in a dilution of 1 in 2,000 only. The oil does not possess very marked anti-bacterial property.

Local action.—The essential oil has a strong pungent odour and when applied locally, it has an irritant action on the unbroken skin. The skin becomes red and slightly swollen. Undiluted oil, when dropped into the eyes of a rabbit, causes vasodilatation of conjunctiva and swelling of lids with photophobia. There is no local anæsthesia.

Action of cardiovascular system.—

(a) *Blood pressure.*—The action of oil was studied on blood pressure of dogs anæsthetized with phenobarbitone. Graded doses of oil, such as

0·01 c.c./kg. to 0·05 c.c./kg. given intravenously produced immediate fall of blood pressure with gradual rise but not to the original level. In atropinized dogs and dogs with vagi cut, a similar fall in B.P. was observed.

(b) *Blood vessels*.—The intestinal volume increases with fall in B.P. but later returns to normal.

Spleen volume.—With fall in B.P., the spleen volume decreases but soon returns to normal.

Leg volume.—No effect.

(c) *Action on heart*.—Myocardiographic experiments in dogs anæsthetized with phenobarbitone showed diminution of amplitude and rate of auricles and ventricles, with doses such as 0·02 c.c./kg. to 0·05 c.c./kg. given intravenously. With smaller doses, the effect soon passes off but larger doses have a more permanent depressant effect.

Action on small intestines.—The effect of oil on the movements of small intestines was studied on dogs anæsthetized with phenobarbitone by balloon method. With graded doses, such as 0·01 c.c./kg. to 0·05 c.c./kg., the normal movements are inhibited.

Isolated intestines.—The action of essential oil was studied on the strips of rabbits ileum in a Dale's bath. The oil inhibits the normal movements of the gut in a diluton of 1 in 80,000.

Action on respiratory system.—The action of oil on the respiration of dogs anæsthetized with phenobarbitone was studied. With doses from 0·01 c.c./kg. to 0·05 c.c./kg. the rate of respiration increased and amplitude decreased. After initial stimulation, however, the rate fell below normal. It showed that the oil has bronchodilator effect. Detailed investigations are in progress and will be reported later on.

Melia azedarch Linn. (vernacular—Bakaya-drek).—A tree which attains the height of 40 feet and has a short erect trunk and broad crown is commonly cultivated in India as an ornamental shade tree. It also grows wild in the sub-Himalayan tract at altitude of 2,000 ft. to 3,000 ft. above sea-level. It was probably introduced into India by Mohammedans and is, therefore, commonly known as Persian lilac. The various parts of the tree are reputed to have the same therapeutic values as those of neem tree. According to Ayurveda the root is acrid, bitter and anthelmintic; it removes tumours, relieves pain in the heart and useful in vomiting, leucoderma and blood impurities.

The Persian lilac has been used by the Arabs and Persians for many years past. The root-bark, fruit, flowers and leaves are considered to be hot and dry and to have deobstruent and resolvent properties. The flowers and leaves are applied as a poultice for nervous headaches. The juice of the leaves administered is said to be anthelmintic, diuretic and emmenagogue and is thought to relieve old cold swellings. In the Punjab, the seeds are prescribed in rheumatism. In Bombay, strings of the seeds are suspended overdoors and verandahs during the prevalence of epidemics to avert the diseases. In America, a decoction of the leaves has been employed in hysteria and is believed to be astringent and stomachic. The leaves and bark are used internally and externally in leprosy and scorfula, while a poultice of flowers is believed to have anthelmintic properties and to be a valuable remedy in eruptive skin diseases. In Indo-China the kernel of the fruit is prescribed in certain forms of fevers and urinary troubles. The fruit

of the plant which is mostly used in the treatment contains an amorphous bitter principle bakayanin, bakaynic acid and sterole.

Bakayanin $C_{21}H_{34}O_4$ is non-glucosidic bitter principle which melts indefinitely at $85^\circ C.$ to $118^\circ C.$ and is bitter in dilutions up to 1 in 100,000. Bakayanic acid is a brown semi-solid acidic product which is also bitter in taste. The sterol melts at $137^\circ C.$ and is identical with sterol isolated from neem blossoms. In spite of the fact that the plant is used so extensively, no pharmacological investigation of the plant has so far been carried out. A preliminary pharmacological investigation of bakayanin yielded the following results :—

1. *Local action.*—A ten per cent suspension of drug in water when applied locally to the unbroken skin of the rabbits does not produce any irritant effect. It has no local action on the mucous membrane and on tissues when injected subcutaneously and intramuscularly.

2. *Action on bacteria.*—The bactericidal action of bakayanin was studied on *Staph. aureus*, *E. coli*, *S. typhosum*, *C. inaba* and *Sh. flexner*. The growth of these organisms was not inhibited at all even in a concentration of 1 in 4,000.

Toxicity.—The acute toxicity to intraperitoneal injection of bakayanin was studied in groups of six albino rats, weighing 80 g. to 120 g. The drug was made into an emulsion with gum acacia and then administered. The D_{50} as calculated by Karber's method is 43.9 mg./kg.

Action on blood pressure.—Dogs anæsthetized with phenobarbitone without morphine were used in this study. With graded doses, such as 20 mg./kg. to 80 mg./kg. given intravenously, there is no effect on blood pressure. With a dose of 100 mg./kg. slight fall in blood pressure is produced which returns to normal within five minutes.

Action on respiratory system.—The action on respiration was studied on dogs anæsthetized with phenobarbitone. With graded dose, such as 20 mg./kg. to 60 mg./kg., there is no effect on respiratory movements. Doses above 80 mg./kg. produce slight increase in rate and amplitude of respiratory movements. The respiration returns to normal after sometime.

Action on small intestine.—The action of the drug on the small intestine was studied on dogs anæsthetized with phenobarbitone. Doses up to 100 mg./kg. have no effect on tone and rythm of the intestinal movements.

Intestinal volume.—No effect.

Spleen volume.—There is decrease in spleen volume. Detailed investigations are in progress.

Investigations completed.—The work on the following two plants was started last year and has been continued during the year under review :—

1. *Acorus calamus* (vernacular—Bach).—

Action on bacteria.—The bactericidal properties of the oil were tested on *Staph. aureus*, *E. coli*, *S. typhosum*, *C. inaba* and *Sh. flexner*. 0.5 c.c. of various concentrations of the oil were added to 9.5 c.c. of the nutrient broth at pH 7.2, which was inoculated with 5-mm. loopful of 24-hour broth culture. The tubes were incubated at $37^\circ C.$ for 24 hours broth culture. The growth of cultures of *E. coli*, *S. typhosum* and *C. inaba* was inhibited in concentrations up to 1 in 1,000 and that of *Staph. aureus* and *Sh. flexner* was inhibited up to 1 in 500 dilution only.

Action on Paramæcia caudatum.—In a concentration of 1 in 200 the essential oil kills all *Paramæcia* instantaneously. In dilutions of 1 in 200 to 1 in 10,000 the movements of the ciliate become sluggish and all die in two to six minutes. In higher dilutions of 1 in 15,000 the movements first become sluggish but after a few minutes the ciliates become quite normal.

Isolated heart.—In the perfused isolated heart of the rabbit the essential oil has a depressant action. With dilutions from 1 in 10,000 to 1 in 20,000 the amplitude and rate of auricular and ventricular contractions decrease. Weak concentrations of the essential oil, i.e. approximately 1 in 30,000 to 50,000, have slight effect. The heart recovers to normal condition when perfused with normal solution.

Isolated intestines.—The action of essential oil on normal and spastic segments of rabbit ileum was determined in Dales bath. The oil in approximate dilutions of 10^{-4} inhibited completely the normal movements. In a dilution of 5×10^{-5} it produced relaxation of contractions produced by acetylcholine. The contractions produced by barium chloride were also antagonized with a dilution of 10^{-4} .

Conclusions.—The essential oil appears to have weak antiseptic properties. It seems to share the carminative property of other essential oils and has an anti-spasmodic action, inhibiting the excessive peristaltic movements of the intestines. The effects of oil on the cardiovascular and respiratory systems are not marked and, therefore, not of much importance from therapeutic point of view.

2. *Curcuma zedoaria* (vernacular—Kachur).—

Action on Bacterial.—The bactericidal properties of the oil were tested on *Staph. aureus*, *E. coli*, *S. typhosum*, *C. inaba* and *Sh. flexner*. The growth of cultures of *Staph. aureus* and *C. inaba* was inhibited in concentrations up to 1 in 1,000. The growth of cultures of *Sh. flexner*, *S. typhosum* and *E. coli* was inhibited in dilutions up to 1 in 500 only.

Action on paramæcia.—In concentrations of 5×10^{-3} the *paramæcia* are killed within a few seconds. In 5×10^{-4} dilutions, the ciliates become sluggish and all die within three minutes. In dilutions of 10^{-4} the movements become sluggish but after two to three minutes the ciliates become normal. Dilutions of 6×10^{-5} show no effect.

Isolated heart.—The action of the oil was studied on isolated heart of rabbit perfused with Ringer-Lock solution. In a dilution of 1 in 5,000 there is a marked depression of the heart, both amplitude and rate decrease. The heart recovers when perfused with Ringer-Lock solution. With a dilution of 10^{-4} gradual decrease in rate and amplitude occurs.

Isolated intestines.—The effects of essential oil on normal and spastic segments of rabbits ileum were studied in a Dales bath. The oil was added to the bath containing oxygenated tyrodes solution. It produces relaxation of normal and contracted segments.

B. ACTION OF ANTIBIOTIC AND OTHER PRINCIPLES FROM INDIGENOUS DRUGS. in vitro STUDIES OF THEIR BACTERICIDAL PROPERTIES.

1. *Cassia absus* Linn. (vernacular—Chaksu).—It is an erect, sparingly branched, annual, 15 cm. to 45 cm. high leaves, compound long petioled, and grows throughout India, Ceylon, tropical Asia, Australia and Africa.

The seeds are bitter and cooling, and are used to cure diseases of the eye, bowel disorders, bronchitis and skin diseases. An extract is made from them to purify the blood. They are also employed in mucous disorders.

Siddiqui and Ahmed (1935) have isolated an alkaloid chaksine having the formula $C_{12}H_{21}O_2N_3$. The wide medicinal use of *Cassia absus* has prompted us to study the anti-bacterial properties of the alkaloid. Heatley's cup-plate and serial-dilution methods were employed and the activity of chaksine sulphate tested against *Staph. aureus*, *E. coli* and *S. typhosum* organisms.

It was observed that chaksine sulphate inhibited the growth of *Staph. aureus* in a dilution of 1 in 10,000 but a dilution of 1 in 500 was necessary to obtain inhibition of *E. coli* and *S. typhosum*.

A 1 in 500 dilution gave a zone of inhibition of 14.5 mm. against a culture of *Staph. aureus*, whereas a dilution of 1 in 100 gave no zone of inhibition against *B. typhosum*.

Toxicity.—The solution when injected subcutaneously to a group of rabbits produced vigorous shivering followed by paralysis of the hind legs and rapid death in a few minutes. Minimum lethal dose for rabbit was found to be 80 mg. per Kgm. body-weight. Further work is in progress.

2. In vitro study of tuberculostatic properties of *Allium schænoprasum* (Chives, vernacular—Kashmiri-Pran).—

This is extensively cultivated in Kashmir and mainly used as a condiment. The bulbs contain an essential oil which possibly contains an antibiotic principle which was obtained by steam-distillation and alcoholic-extraction method for experimental purposes.

Anti-bacterial properties.—The anti-bacterial properties of the oil were tested on *M. tuberculosis* (B_{19-4} human, H_{52} human, B_{19-3} bovine, B_{19-1} avian, H_{52} Rs. stains), *S. aureus*, *S. typhosum*, *E. coli*, *C. inaba*, *B. friedlander* and *B. proteus*. 0.5 c.c. of various concentrations of the substance was added to 0.5 c.c. of the nutrient broth, pH 7.2, which was inoculated with 5-mm. loopful of 24-hour broth culture. The tubes were incubated at 37°C. for 18 hours. The anti-tubercular activity was determined by adding 0.5 c.c. of the drug to 4.5 c.c. of Youman's medium. The tubes were inoculated with 0.01 mg./c.c. of the culture and incubated at 37°C. for 14 days and results read.

The oil inhibits the growth of human strains completely in a concentration of 2.5 mg./c.c. and partially in a concentration of 1.25 mg./c.c., while the growth of 0.1 c.c. of seven-day culture of H_{52} Rs. streptomycin-resistant strain is inhibited in concentration of 1.25 μ g. Similarly, the growth of bovine and avian strains is inhibited partially in a concentration of 2.5 μ g./c.c. and completely in a concentration of 5 μ g./c.c.

The oil inhibits the growth of *Staph. aureus* in a concentration of 200 μ g./c.c., while the growth of *E. coli*, *S. typhosum*, *C. inaba*, *B. friedlander*, *B. proteus* is not inhibited at all even at 200 μ g./c.c. Thus, the oil seems to have a selective action on *M. tuberculosis*.

Conclusion.—The oil and possibly antitubercular principle in it obtained on steam-distillation and also on alcoholic-extraction from *Allium schænoprasum* known as Pran in Kashmir has a marked anti-tubercular activity. This

anti-tubercular activity is slightly less than that of streptomycin for streptomycin-sensitive strains. Streptomycin-resistant strain is as equally susceptible as streptomycin-sensitive one. The action of the oil seems to be specific for tubercule bacilli as the growth of other organisms with the exception of *Staph. aureus* is not inhibited even with a concentration of 200 µg./c.c.

3. *In vitro study of combined action of chloromycetin and ptergospermin.*—*Ptergospermin* (Marium *et al.*) is the active principle isolated from the roots of *Moringa ptergosperma*. It has marked anti-bacterial spectrum against Gram-positive and Gram-negative organisms. The experiments were performed to test the anti-bacterial effect of ptergospermin in combination with chloromycetin against *E. coli*, *S. typhosum*, *B. paratyphosum* and *M. tuberculosis*.

Nutrient broth of pH 7.2 was used after adding the required amount of chloromycetin and ptergospermin the final volume came to 10 c.c. Five-mm. loopful of 18-hour broth culture was used and the tubes incubated at 37°C. for 18 hours and results read.

For acid-fast bacteria Youman's.—The tubes were inoculated with 0.01 mg./c.c. of culture, incubated for 14 days and results read.

Chloromycetin and ptergospermin when used alone inhibit growth of *E. coli* in a dilution of 0.003 mg./c.c. and 0.04 mg./c.c., respectively. When, however, both are used in combination, ptergospermin in combination with non-inhibitory concentration, chloromycetin can inhibit growth completely in concentration of 0.0025 mg./c.c. and partially up to 0.00125 mg./c.c.

Chloromycetin inhibits the growth of *S. typhosum* in a dilution of 0.001 mg./c.c., 0.04 mg./c.c. of ptergospermin inhibit completely and 0.02 mg./c.c. partially the growth of the test culture. Ptergospermin used in combination with 0.0009 mg./c.c. of chloromycetin can inhibit the growth completely in a dilution of 0.01 mg. and partially in a concentration of 0.005 mg./c.c.

The inhibitory concentrations of chloromycetin and ptergospermin when used in combination with non-inhibitory concentration of chloromycetin (0.006 mg./c.c.) can inhibit growth completely in a concentration of 0.01 mg./c.c. and partially up to a concentration of 0.00125 mg./c.c.

Ptergospermin which does not inhibit growth, when used in combination with 0.005 mg./c.c. of chloromycetin can inhibit the growth completely of streptomycin-resistant strain of tubercule bacilli in a concentration of 0.01 mg./c.c. and partially up to a concentration of 0.0006 mg./c.c.

3. Inquiry into the isolation of the active principles of the plants of *N. O. Dioscoreaceæ* under Dr. R. N. Chakravarti at the School of Tropical Medicine, Calcutta.

Steroid sapogenins are now-a-days playing an important rôle as starting material for the large-scale preparation of cortisone. Some of these sapogenins occur as glycosides in the yams of dioscorea plants and are obtained by isolation of the crude saponin from the yams followed by hydrolysis of the product with alcoholic hydrochloric acid. The chief sapogenin of dioscorea yams is diosgenin which is the best starting material

available for the preparation of cortisone and a number of other useful steroid hormones. Although at C-11 in ring C, cortisone has an oxygen function which is not present in diosgenin, a method has recently been discovered for the introduction of the same in ring C of progesterone by biological oxygenation in a single step, and as progesterone can be economically prepared from diosgenin, this method provides a practical route for the preparation of cortisone from the latter.

In the year under review considerable progress has been made in the matter of chemical investigation of the yams of Indian dioscorea plants and some extremely encouraging results have been obtained. Chemical investigation of the yams of nine new dioscorea plants was carried out for the isolation of useful saponin. Three of the yams could not be identified properly. The remaining six are : (11) *D. hispida* (12) *D. prazeri*, (13) *D. nummularia*, (14) *D. glabra*, (15) *D. bellophylla*, and (16) *D. aculeata*. The remaining parts of the works on (3) *D. pubera*, (*D. anguina*) (5) *D. bulbifera*, (6) *D. oppositifolia*, (7) *D. sativa*, (8) *D. esculenta*, (9) *D. deltoidea*, and (10) *D. Wallichii* were also carried out. The yams were collected from various parts of India, mostly through the Forest Offices of the localities. It has been found that two of the Indian dioscorea plants, *D. deltoidea* and *D. prazeri*, are excellent sources for diosgenin. Both these plants grow wild in hilly parts of the country and the yams are used by the hill tribes for various purposes, e.g. (1) for washing wool and silk, (2) as an insecticide to remove lice from hair, and (3) as a fish poison for catching fish. The yields of diosgenin from *D. deltoidea* (3.35 per cent) and *D. prazeri* (2.1 per cent) appear to be far greater than those obtained from various other sources as recorded in the literature. This is extremely important in view of the great demand for diosgenin for the preparation of cortisone and some of the other useful steroid hormones.

D. pubera (*D. anguina*).—The sample described as *D. anguina* in the previous report was later identified as *Pueraria tuberosa*. *D. pubera* (*D. anguina*) was later collected. It did not appear to contain any saponin.

D. bulbifera.—The crude saponin fraction from the yams of this plant on hydrolysis gave a very small amount of solid mass which appeared to be a bad mixture of saponin. The constituents could not be separated due to the poor yield of the crude saponin fraction.

D. sativa.—It appeared to contain traces of saponin. On hydrolysis for the isolation of saponin no crystalline product could be isolated.

D. esculenta.—The crude saponin isolated from the yams of this plant on hydrolysis with five per cent alcoholic hydrochloric acid gave a crystalline mass which was found to be chiefly diosgenin, m.p. 197°C., (α) D-128.3°. It appeared to contain two other saponin in traces. The yield of diosgenin was found to be 0.17 per cent. With acetic anhydride it gave a mono-acetate, m.p. 190°C. to 191°C., and with benzoyl chloride it gave a monobenzoate, m.p. 236°C. The saponin and the above two derivatives were compared with respective authentic specimens kindly supplied by Professor Carl Djerassi of Wayne University, U.S.A., and syntex S. A. Mexico.

D. deltoidea.—It appeared to contain large amounts of saponin. The crude saponin isolated from the yams of this plant, on hydrolysis in the usual manner for the isolation of the saponin, gave a fine crystalline product m.p. 204°C. to 206°C. It was found to be identical with an authentic

sample of diosgenin. Yield of diosgenin was found to be 3.35 per cent. With acetic anhydride it gave diosgenin acetate, m.p. 194° C. to 195° C., and with benzoyl chloride it gave diosgenin benzoate, m.p. 236° C. to 237° C.

D. oppositifolia.—It appeared to contain saponin only in traces. No saponin could be isolated in crystalline form.

D. wallichii.—It appeared to contain traces of saponin. On hydrolysis no saponin could be isolated in crystalline state.

TABLE.

Yields (per cent) of various fractions obtained from the yams of plants by extraction with various solvents.

Plants			Petroleum ether	Ether	Chloroform	Ethyl acetate	Alcohol
<i>D. pubera</i> (<i>D. anguina</i>)	0.69	1.05	0.31	1.13	4.65
<i>D. oppositifolia</i>	0.58	0.55	0.32	1.12	2.91
<i>D. wallichii</i>	0.63	0.35	0.37	2.11	4.28
<i>D. hispida</i>	0.41	0.67	0.95	1.18	0.94
<i>D. prazeri</i>	0.85	2.31	0.93	7.63	9.51
<i>D. nummularia</i>	0.53	0.78	0.54	1.35	3.23
<i>D. glabra</i>	0.88	0.49	2.53	2.34	4.81
<i>D. bellophylla</i>	0.80	0.37	0.56	2.15	10.45
<i>D. aculeata</i>	0.66	0.32	0.28	0.92	0.86
<i>Unidentified species.</i>							
No. 1	0.71	0.98	0.24	1.15	10.30
No. 2	0.67	0.66	0.28	2.92	1.44
No. 3	0.53	0.49	0.27	1.44	8.13

D. hispida.—The alcoholic fraction was found to contain no traces of saponin and the petroleum ether, ether and particularly chloroform fraction showed the presence of sufficient amounts of alkaloid. The poisonous character of this yam is not due to any saponin but is due to the alkaloid fraction. The chief alkaloid of this plant is dioscorine (Boorsma, 1894) Meded units Lands Plant ; Schutte (1897), *Chem. Zentr.*, **ii**, p. 130 ; Pinder, A. R. and Robinson, R. (1952), *Jour. Chem. Soc.*, p. 2236).

D. prazeri.—The alcoholic fraction was found to contain considerable amounts of saponin. The ethyl-acetate fraction was also found to contain saponin but in small amounts only. The crude saponin isolated from the yams of this plant on hydrolysis with five per cent alcoholic hydrochloric acid gave a fine crystalline product, m.p. 204° to 206°, (α)D-129°. It was found to be identical with diosgenin by comparison of the mixed m.p. with authentic sample of diosgenin. With acetic anhydride it gave diosgenin acetate, m.p. 194°C. to 195°C. and with benzoyl chloride it gave diosgenin benzoate, m.p. 236°C. to 237°C. The yield of diosgenin was found to be 2.1 per cent.

D. nummularia.—Only the alcoholic fraction was found to contain traces of saponin.

D. glabra.—The alcoholic fraction was found to contain traces of saponin.

D. bellophylla.—Only the alcoholic fraction was found to contain traces of saponin.

D. aculeata.—None of the fractions appeared to contain any saponin.
Unidentified species :

No. 1—None of the fractions was found to contain any saponin.

No. 2—None of the fractions was found to contain any saponin.

No. 3—The alcoholic fraction was found to contain saponin in small amounts. Detailed work was not possible due to want of material.

An abstract of a paper meant for symposium on 'Indigenous Drugs and Insecticides' held at Bombay in August 1953, has been published. The full paper is to appear in the *Proceedings of the National Institute of Sciences of India*. A paper is in under publication in the August issue of the *Indian Medical Gazette*. Another paper has been accepted for publication in the *Journal of the Indian Chemical Society*. A short communication is to appear in the October issue of the *Bulletin of the School of Tropical Medicine*, Calcutta.

4. Inquiry on pharmacological action and biological assay of adrenal cortical hormones under Dr. B. Mukerji at the Central Drug Research Institute, Lucknow.

As it is claimed by some workers that the 'mouse eosinophil test' method of assaying 11-oxycorticoids of the adrenal cortex is the most sensitive one, attempts were made to standardize this method during the period under review. Some time was spent in perfecting the technique of adrenalectomy. In the initial stages, several experimental animals were lost as a result of the operational shock.

As a first step, efforts were made to draw a dose-response curve by injecting varying doses of cortisone acetate in adrenalectomized mice and rats.

Total eosinophil counts of some normal mice was made by the total w.b.c. count along with differential count. This procedure was, however, time-consuming and the results inconsistent. It was, therefore, discarded.

The total count of circulating eosinophils by the *direct method* was then attempted. In the direct method of counting, the blood is diluted with a special fluid : 10 c.c. of one per cent phloxine solution, 1 c.c. di-ethylene glycol, 30 c.c. distilled water and 7 c.c. acetone. In this fluid all the r.b.c. are destroyed and all the different types of w.b.c. except the eosinophils form hazy images in the background. Phloxine imparts a distinctive red stain to the eosinophil granules and thus it becomes comparatively easy to count them. In all 28 experiments were carried out, using 6 μ g. of cortisone acetate. The average percentage decrease of circulating eosinophils was found to be 65 ± 20 .

At first, cortisone-acetate suspension in normal saline was used but the dosage could not be regulated as the crystals settled down during the administration of the drug. Cortisone-acetate crystals were then dissolved in sterile olive oil. In room temperature this solution lost its potency within 15 days. To avoid this, a fresh sample of cortisone acetate in oil was kept in the refrigerator. This method of preservation also failed to retain the potency for more than 22 days. This difficulty is standing in the way of collection of a large number of data for statistical analysis.

Further investigations are in progress to avoid these handicaps and to get a series of data which could be considered sufficiently accurate to be acceptable for bioassay purposes.

5. Inquiry on the effects of cardio-active drugs, such as digitalis, strophanthine, etc. and related compounds, on various metabolic reactions involved in muscle contraction under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta.

During this period work has been carried out mainly along the following lines :—

- (a) Effect of action on the excitability and contractibility of muscle tissues of frogs.
- (b) Effect of cardiac drugs on muscle contraction.
- (c) Effect of cardiac drugs on isolated frog's heart.
- (d) Effect of cardiac drugs on glycerinated muscle fibres.

(a) Effect of actions on muscle excitability and muscle contraction.

Muscle excitability.—Whenever any muscle tissue, either of a frog or of a rabbit, is exposed to a very highly concentrated solution of KCl the muscle, almost instantaneously, loses its excitability, i.e. it does not respond any more to electrical stimulation. If, on the other hand, these concentrated solutions are replaced by lesser concentrated solutions, the time required for the complete loss of excitability is greatly increased, with extremely dilute solutions, however, the muscle gradually and very slowly loses this property and finally becomes completely inexcitable. Such muscles which have lost their excitability, have been found to recover their lost property progressively when repeatedly washed with Ringer.

When KCl is replaced by NaCl we hardly find any difference in their behaviour. Here also the muscle loses its excitability—the time required, as usual, depends upon the concentration of the salt solutions. Calcium plays an important rôle in biological phenomena. In CaCl_2 solution too, the muscle loses its excitability. All our findings suggest that any electrolyte which can bring about a change in the membrane potential will be sufficient to cause the muscle inexcitable. This destruction of excitability by different electrolytes is, in no way, connected with the contractile mechanism because such inexcitable muscle could be made to contract by various chemical agents. The destruction of excitability is, therefore, intimately associated with the membrane potential alone.

Muscle contraction.—Muscle shows a pronounced contracture when an excess of K^+ is applied ; the magnitude of this diminishes progressively as the concentration of KCl solution is gradually decreased, until finally it does not contract any more. At 0.03 M salt concentration the muscle does not contract at all, but even then it loses its excitability. In straited muscle K^+ can, also induce contracture.

Such effects of KCl cannot be simulated with NaCl. In NaCl solutions (0.5 M to 0.03 M) the muscle does not contract at all.

Protoplasm of muscle cells is extremely sensitive to Ca^{++} ; with $CaCl_2$ solutions the muscle gradually and very slowly goes to contracture and remains in that form. In each and every case the muscle contracts after a certain period of time (latent period the magnitude of which increases in time) as the concentration of the salt solution is decreased. Other divalent ions, such as Sr, Ba, can simulate this effect of Ca, but they are extremely toxic to animal tissues. This is perhaps the main reason why we do not find other divalent ions except Ca^{++} in our body-fluids and in animal tissues.

(b) *Effect of cardiac drugs on muscle contraction.*

When sartorius or gastronemius muscle of frog is exposed to solutions of cardiac drugs of the digitalis series, the muscle does not contract ; but it loses its another very important property, i.e. its excitability. This loss of excitability is not perhaps due to the drug itself, because solutions of these drugs are usually prepared in normal saline and due to the presence of this electrolyte the membrane potential is changed. This was further verified by adding graded amounts of NaCl to a series of solutions of digitalin and by measuring the time required by the muscle, in each case, to lose its excitability. Surprisingly enough, the time required for the complete loss was found to be higher, the lower the concentration of the salt concentration. This loss of excitability should not, therefore, be attributed to the action of the drug.

(c) *Effect of cardiac drugs on isolated frog's heart.*

When isolated frog's heart is perfused with solutions of these drugs, the heart stops in systolic contracture. These actions could, however, be removed by continuous washing with Ringer. In some of the cases the perfused heart requires a prolonged washing with Ringer, in others a few times of washing are sufficient to remove the cardiac effects. One thing is, however, common in all such cases, i.e. the heart revives its original condition and beats in a normal fashion. It is quite natural, therefore, to infer that the binding of the drug with the muscle proteins or the adsorption of the drug on the membrane surface is not irreversible. When the drug is more firmly fixed, then alone it requires continuous washings for a longer period of time.

The stoppage of the isolated heart is believed by some workers to be due to their action on cardiac muscle. Others think that their effect is mainly due to their action on the vagus nerve-endings. Usually these drugs are known to affect parasympathetic nerves as a result of which acetylcholine is liberated and ultimately brings about the stoppage of the

movements of the perfused heart. We have already mentioned that neither the sartorius nor the gastronemius muscle contracts under the influence of these drugs. The question then arises as to what prevents these muscles to contract. Heart muscle consists of a large number of nucleated cross-striated tissues ; unlike the cells of skeletal muscle the cardiac muscle fibres have no sarcolemma which separates one fibre from the other. In heart muscle they are joined with one another and in this way they establish continuity from fibre to fibre. In skeletal muscle a number of separate units are bound together ; otherwise there is no fundamental difference between the two types of muscles, if so, we would then expect these muscles to contract, but this does not happen. Where then lies the difference ? This problem is therefore of fundamental character, and the answer is still unknown. Further work along this line is in progress.

(d) *Effect of cardiac drugs on glycerinated muscle fibers.*

From rabbit psoas muscles, glycerinated muscle fibers have been prepared according to the method of Szent-Györgyi by keeping them in 50 per cent glycerine solution at 0°C. for 24 hours and then at -20°C. to -20°C. for two days. These fibres are separated to smaller bundles of fibres and thoroughly washed with glass-distilled water cooled to 0°C. The fibres are then put into a series of dishes containing 3 c.c. of 0.16 M KCl, 1 c.c. of veronal buffer of pH 7.4 and graded amounts of digitalin. Such muscle fibres did not contract at all. From these results we can conclude that none of these drugs has any action on the contractile element actomysin. These drugs have no action on myosin or actin, the two contractile proteins of muscle. It follows, therefore, that these drugs can exert their action as long as the membrane is there.

6. Inquiry on indigenous drugs under Dr. B. C. Bose at the M. G. M. Medical College, Indore.

During the period under review the first part of the work of the scheme, viz. survey of active principles of some of the official and semi-official varieties of medicinal plants and herbs, growing in Madhya Bharat, were taken up in hand for investigation. This part of the work has practically been completed and the findings are contained in the present report.

For the collection of plants and herbs, the help of the Forest Department of Madhya Bharat was taken and through their courtesy samples of crude drugs, growing in and around the area of Indore, were procured and submitted to the methods of analysis as prescribed in B.P., B.P.C. and I.P.L.

The particulars of these drugs and their analytical values are given below :—

Acacia arabica.—It is available in fairly large quantities all over Madhya Bharat. The gum collects as round or long cylindrical tears of varying sizes, colourless or reddish, odourless, translucent and brittle. They conformed to the identification, microscopical and chemical tests, viz,

lead sub-acetate, benzidine colour reaction, Starch, dextrin, tannin, etc., as prescribed in B.P. for gum acacia. Further, the sample on repeated analysis, showed the following chemical constants (Table I) :—

TABLE I.

Tests applied	Observations, per cent	B.P. requirements, per cent
Agar and tragacanth, starch, dextrin and tannin	Absent	Absent
Loss on drying at 100° C.	Average—13 Range—12 to 14	Not more than 15
Total ash content	Average—1·8 Range—1·4 to 2	Not more than 5
Acid-insoluble ash content	Average—0·24 Range—0·23 to 0·25	Not more than 0·5

Citrullus colocynthis.—The fruit, which grows in various parts of Madhya Bharat, was obtained in dry condition and identified both macroscopically and microscopically. The findings of chemical analysis are given in Table II :—

TABLE II.

Tests applied	Observations, per cent	B.P. requirements, per cent
Acid-insoluble ash ...	Average—3·5 Range—3 to 4	Not more than 6
Light petroleum (boiling-point 50° to 60° of soluble matter) ...	Average—1·2. Range—1 to 1·4	Not more than 3

Santalum album.—The tree grows over large areas of Madhya Bharat, such as Indore, Nemar, etc., and is not being commercially exploited in the State at present. Suitable samples of the white variety of sandal-wood was procured through the Forest Department of Madhya Bharat Government. The wood is yellowish-white in colour, presenting on transverse section light and dark concentric zones, with characteristic odour. The heart-wood was submitted to steam-distillation for the purpose of extraction of oil.

The percentage yield of the oil and its physical constants are contained in Table III :—

TABLE III.

Tests applied	Observations, per cent	Standards, per cent
Percentage of oil extracted ...	Average—3 Range—2·8 to 3·2	2·5 to 6 (Chopra).
Refractive index ...	1·51	1·505 to 1·510 (B.P.C.)
Weight per c.c. ...	0·978 g.	0·971 to 0·983 g. (B.P.C.)

The other values, such as optical rotations, ester- and free-alcohol contents are being determined and will be incorporated in the final report to be submitted on completion of the work.

Holarrhena antidysenterica.—The tree is reported to be growing in various parts of Madhya Bharat in wild states. One sample of bark was collected through the courtesy of the Forest Department and another sample by ourselves from the Indore area. In both these cases the trees selected for the collection of the bark were over ten years of age. The barks conformed to the identification particulars. They were curved, channelled, of requisite thickness, with greyish-brown outer surface and longitudinal striations on the inner surface and bitter in taste.

Chemical analysis revealed the following (Table IV) :—

TABLE IV.

Tests applied	Observations		I.P.L. specification, per cent
	Sample A, per cent	Sample B, per cent	
Acid-insoluble ash ...	·82	·75	Not more than 1
Total alkaloids ...	2·1	2·3	Not less than 2

Adhatoda vasika.—The plant grows abundantly in Indore area and also in varying quantities in other parts of Madhya Bharat. The leaves—lanceolate, of varying sizes, one-nerved, petiolate, acuminate with reticulate venations, acute bases, long opposite branches, leathery surface with hair, and swollen nodes.

The leaves were collected and dried at 40° C. The extraction of the alkaloid was carried out in the following way :—

The dried leaves were extracted with a mixture of two parts of solvent ether and one part of chloroform. The alkaloid was precipitated by adding strong solution of ammonia and left overnight. Chlorophyll, etc., was removed by adding water and the supernatant fluid decanted. The alkaloid was extracted by acid, such as hydrochloride, washed with

chloroform and precipitated by ammonia. It was finally extracted with chloroform which was evaporated and the residue dried at 100°C. till constant weight was obtained.

The findings are given in Table V.

TABLE V.

Alkaloid contents		Chopra's observations
Sample A, per cent	Sample B, per cent	Up to 0.25 per cent
0.24	0.25	

Ægle marmalos (Bæl).—It grows in plenty in almost all over Madhya Bharat and is used by the indigenous practitioners and also for the purpose of preparations of jams (Murrabba). The sample, obtained locally, was half-ripe and presented the following characteristics, conforming to I.P.L. requirements :—

Size of the fruit	2" to 4" in diameter
Thickness of the pericarp	2 mm. to 4 mm. in diameter
Water-soluble mucilaginous substance	20 per cent.

Datura alba.—The plants, which were collected for investigation purposes, presented the following characters. They were about six months old, 2' to 3' in height, with green leaves, white flowers and green fruits.

Stem.—Cylindrical, slightly furrowed and hairy.

Leaves.—Ovate, hairy, with unequal bases and glabrous margins.

Flowers.—With characteristic 5-toothed calyx, whitish-yellowish corolla, 5 stamens, bicarpellary, syncarpus ovary.

Fruits.—Round, four-walled, covered by stiff emergences and reniform, flat brown seeds.

The analytical findings are given in Table VI :—

TABLE VI.

	Acid-insoluble ash, per cent	Alkaloid contents, per cent
Leaves	2.5	0.06
Seeds	...	0.39

7. Inquiry on the biogenesis of alkaloids of tobacco plants under Dr. B. C. Bose at the M. G. M. Medical College, Indore

In the period under review the first part of the scheme, i.e. to trace out the different potent precursors which aid in the synthesis of pyridine alkaloids in the tobacco leaves, was undertaken.

DEVELOPMENT OF A MICRO-METHOD OF ESTIMATION OF PYRIDINE ALKALOIDS.

The main plan of work was based on tissue-culture technique in which small portion of the slices of leaves or stems of tobacco plant was cultured in a synthetic medium with and without any probable precursors for biogenesis and naturally in such cases one has to adopt a method by which the nicotine or pyridine alkaloids can be measured to the extent of the minute amount of one part per million.

In the literature, two methods have been prescribed for the estimation of nicotine : (a) Titration method, and (b) silicotungstic method. In both these methods a large amount of tobacco leaves varying from 2.5 g. to 10 g. is required for estimation of these alkaloids and hence in our proposed investigations these methods could not be satisfactorily adopted. It was, therefore, felt necessary to devise some other micro method which will allow to estimate minute amounts of the above alkaloids from a very small quantity of leaves and after some efforts the following method was developed :—

While searching out a suitable method our main aim was to explore a reagent which will produce some colour even at low concentrations of the pyridine alkaloids. From the knowledge of the mechanism of nicotinic-acid estimation by cyanogen-bromide and aniline reagents, it appeared that these reagents may be applied in the estimation of pyridine alkaloids in minute amounts. Moreover, the method for nicotinic-acid estimation by the above reagents is based on the previous observations by Tallantyre, (*Jour. Soc. Chem. Ind.*, **49**, p. 446, 1930) and others, in their classical work on the colour reaction of nicotine and other pyridine alkaloids with the above reagents. So, the cyanogen-bromide and aniline method of nicotinic-acid estimation was applied for the estimation of total pyridine alkaloids in the tobacco plant and the following are the values obtained by this micro-method :—

Comparative study of values obtained by the titration and the new micro-method.— 2.5 g. of dry tobacco leaves, collected from the market and 125 c.c. of one per cent NaOH were taken in a distillation flask and nicotine distilled off by steam and collected in a receiver containing 5 c.c. of N/10 H_2SO_4 . For complete distillation of the alkaloid the distillate was collected up to 500 c.c. Six samples of tobacco leaves were distilled in the above way and three of these were analysed for total nicotine content by titration against N/10 NaOH (each c.c. of N/10 H_2SO_4 consumed representing 16.2 mg. of nicotine). Other three distillates were adjusted to pH 6 by addition of alkali and subjected to colorimetric estimation in the following way :—

To 1 c.c. of the distillate was added 1 c.c. of 50 per cent sodium-acetate buffer, adjusted to pH 7, 2 c.c. of two per cent aqueous aniline solution (freshly prepared) followed by 12 c.c. of cyanogen-bromide solution and mixed well and allowed to stand for ten minutes during which period

maximum intensity of the colour was reached. This was matched against a standard prepared in the same way by taking 1 c.c. of solution containing 100 μ g. of nicotine.

Since the colour developed was too intense, the visual colorimeter was used for comparison and the results presented in Table I show that per cent of nicotine estimated by the titration and cyanogen-bromide methods is almost the same.

TABLE I.

Showing the comparative values of the nicotine estimated by titration and colorimetric methods.

(Figures represent the average of six estimations in each batch).

<i>Batch A</i> — tobacco leaves taken—2.5 g.	Distillate collected to 500 c.c. in 5 c.c. N/10 H_2SO_4	Nicotine estimated by titration method against N/10 NaOH 3.2 per cent.
<i>Batch B</i> — tobacco leaves taken—2.5 g.	Distillate collected to 500 c.c. in 1 c.c. 1 : 1 HCl	Nicotine estimated by visual colorimeter 3.0 per cent.

MICRO-ESTIMATION OF NICOTINE BY THIS METHOD.

As the colour developed by concentration of 100 μ g. to 150 μ g. of nicotine as present in 2.5 g. of tobacco leaves was very intense and could be easily compared by visual colorimeter, the next object was to determine the minimum amount of tobacco leaves the nicotine content of which could be easily estimated by photo-electric colorimeter even for low intensity of colour in such cases.

Small amount of leaves varying from 0.5 g. to 0.04 g. was subjected to steam-distillation as before in presence of NaOH and the distillate collected in a solution containing 1 c.c. of 1 : 1 HCl up to 500 c.c. as before, and this was then analysed in a similar way colorimetrically by the cyanogen-bromide-aniline method. The results below show that the per cent of nicotine estimated by taking only 0.5 g., 0.2 g. and 0.04 g. of leaves fairly agrees with the values previously obtained by taking 2.5 g. of leaves. The results further show that nicotine present even in 40 mg. of leaves can be easily estimated by this method by using photo-electric colorimeter for colour comparison. The colour obtained by taking less than 0.04 g. of leaves was very faint and gave a very low galvanometric deflection. For measuring such small quantities of nicotine the device of 'intensification of colour', described elsewhere, is necessary.

TABLE II.

Showing the values of nicotine in small amounts of tobacco leaves estimated by cyanogen-bromide reagent with photo-electric colorimeter.

Amount of tobacco leaves taken, g.	Nicotine estimated, per cent
0.5	3.06
0.2	2.96
0.04	3.0

PERCENTAGE RECOVERY OF ADDED NICOTINE.

The next approach in the work was to ascertain as to whether the nicotine present in the leaves was fully distilled off and recovered from the distillate.

The following experiments were performed to elucidate this point :—

In one series of experiments small amount of nicotine to the extent of 1 mg. was subjected to steam-distillation and the percentage recovery of this nicotine was estimated by cyanogen-bromide method.

In another batch the same amount of nicotine was mixed with 40 mg. of tobacco leaves and the per cent recovery of the added nicotine in such cases was also determined. The results presented in Table III show that in both these cases the per cent recovery of nicotine is nearly 96 to 98. From this finding it is evident that the method is sufficiently sensitive for estimation of minute quantities of nicotine present in the leaves.

TABLE III.

Showing the recovery of added nicotine by cyanogen-bromide method.

(The values represent the average of six experiments).

Batch number	Tobacco leaves, mg.	Nicotine added, mg.	Recovery of added nicotine, mg.	Percentage recovery
A	<i>Nil</i>	1	0.98	98
B	40	1	0.96	96

Minimum amount of distillate to be collected for nicotine estimation.—In all the above cases, 500 c.c. of the distillate was collected to obtain the maximum recovery. Since such amount of distillate involved a longer period, it was, therefore, thought advisable to ascertain the minimum volume of the distillate which requires to be collected for the maximum recovery of nicotine. The experiments for this purpose were arranged in the following way :—

Different batches, containing 1 mg. of nicotine alone and in combination with 40 mg. of tobacco leaves, were subjected to steam-distillation as before and the distillates collected in different volumes from 100 c.c. to 500 c.c. for each batch of experiment and the per cent recovery of nicotine in every distillate was then estimated by the above method. The results presented below show that at the volume of 300 c.c. the per cent recovery is nearly 95 to 96 and this remains constant even when the distillate is collected up to 500 c.c. At distillate volumes of 100 c.c. and 200 c.c. the per cent recovery was 60 to 70. It, therefore, appears that for maximum recovery of nicotine from small quantities of leaves a minimum of 300 c.c. of distillate should be collected (Table IV).

TABLE IV.

Showing the amount of nicotine recovered in different volumes of distillate.

(The figures for each batch represents the average for three estimations).

Batch number	Tobacco leaves, mg.	Nicotine added, mg.	Volume of distillate collected, c.c.	Recovery of nicotine, mg.	Percentage recovery
A	<i>Nil</i>	1	200	0·62	62
B	<i>Nil</i>	1	300	1	100
C	40	<i>Nil</i>	200	0·94	78
D	40	<i>Nil</i>	300	1·20	100
E	40	<i>Nil</i>	400	1·14	95
F	40	<i>Nil</i>	500	1·20	100
G	40	1	200	0·65	65
H	40	1	300	0·98	98
I	40	1	400	0·97	97
J	40	1	500	0·96	96

INTENSIFICATION OF THE COLOUR DEVELOPMENT.

While estimating nicotine at low concentration below 2 μ g. per c.c., it was observed that the galvanometric deflection of seven to eight divisions only was produced. Since the multiflex galvanometer which could increase the range of deflection and thus also the accuracy of results, was not available, it was considered necessary to increase the intensity of colour by adding an extra quantity of 2 mg. of nicotine to both the unknown as well as the standard solutions. This procedure gave more satisfactory results.

From the survey of the results detailed above, it appears that cyanogen-bromide and aniline methods, as applied for nicotinic-acid estimation, can be utilized for the estimation of nicotine. This method is not specific for nicotine only but also for all other pyridine alkaloids present in the tobacco plants. This can be easily applied for the estimation of different pyridine alkaloids after their separation by paper-chromatography. Investigations are being made in this line to utilize this method for estimation of nor-nicotine, anabesine and other pyridine alkaloids in minute amounts.

Investigation on the probable precursors for biogenesis of alkaloids in tobacco plants.—Side by side with the establishment of the micro-method technique for all the investigations detailed above, the study of the possible precursors for biogenesis of nicotine and other pyridine alkaloids was also taken up. Three varieties of seeds : *Nicotiana tabacum*, *Nicotiana glauca*, and *Nicotiana glautinosa* were obtained from the Central Tobacco Committee. The soil of the herbarium was analysed and fertilized with compost. Some seeds of *Nicotiana tabacum* were sown on a small plot of this herbarium soil. When the plants grew well, fifty of them were taken out and transplanted at uniform distances in a bigger plot of land suitably prepared in advance for this purpose.

Due care was taken about watering and other particulars for their growth. When they were one month old from the date of transplantation, the young leaves of 50 mg. to 100 mg. were cultured in synthetic liquid medium containing glucose, nitrates, phosphates, etc., with the precursors 1-arginine and 1-tryptophane. They were incubated at room temperature for a period of 24 hours. A set of culture was also kept as control without any treatment by the above precursors.

TABLE V.

Showing the effect of incubating tobacco leaves with 1-arginine and 1-tryptophane on the nicotine synthesis.

(The figures represent the average of three experiments in each batch).

Batch number	Dose of the precursors in 40 c.c. culture medium, mg.	Percentage of nicotine on wet-weight basis	Percentage increase of nicotine
Control ...	<i>Nil</i>	0·518	...
1-arginine supplement ...	50	0·687	30
1-tryptophane supplement ...	50	0·740	42

The results presented in Table V show that both 1-arginine and 1-tryptophane can stimulate the synthesis of pyridine alkaloids including nicotine in tobacco plants to a considerable extent.

On the basis of this finding from the laboratory-culture technique in synthetic culture work in the medium, work has also been taken in hand to spray 1-arginine and 1-tryptophane on the leaves of a series of plants, growing in the garden, with a view to explore whether this spraying process will increase the nicotine content of the tobacco leaves further.

IV. ABSTRACTS FROM REPORTS OF I.C.M.R. RESEARCH FELLOWS.

1. Biochemical studies on normal blood in Orissa by Dr. J. M. Senapati at the S. C. B. Medical College, Cuttack.

In course of the investigations, it was found that in Orissa, the intake of first-class proteins, fats, vitamins, e.g. thiamin, riboflavin, nicotinic acid, ascorbic acid, and minerals, specially calcium, was low and the hæmatological studies revealed incidence of mild type of nutritional anæmia in normal students. It was necessary to find out the biochemical constituents in blood of apparently healthy individuals in Orissa. Table below presents the summary of findings in respect of the constituents detailed :—

TABLE.

Item	Number of cases	RESULTS :		COMPARATIVE STANDARDS	
		Range, g. per cent	Mean, g. per cent	Datta	Chakravarty
1. Plasma proteins and fractions :					
Total proteins	100 males	5.32–7.60	6.430 \pm 0.075 0.744 \pm 0.053	7.51	7.1
	50 females	5.32–7.50	5.980 \pm 0.091 0.636 \pm 0.064	7.49	...
Albumin ...	100 males	3.10–4.90	4.000 \pm 0.052 0.589 \pm 0.042	4.93	4.5
	50 females	3.10–4.85	3.700 \pm 0.076 0.530 \pm 0.054	4.87	...
Globulin ...	100 males	1.27–3.01	2.100 \pm 0.049 4.488 \pm 0.035	2.58	2.6
	50 females	1.27–3.01	1.820 \pm 0.069 0.485 \pm 0.049	2.66	...
Fibrinogen ...	100 males	0.04–0.80	0.450 \pm 0.025 0.254 \pm 0.018	0.26	...
	50 females	0.04–0.90	0.450 \pm 0.040 0.280 \pm 0.028
Albumin/: globulin ratio ...	100 males	1.27–2.91	2.050 \pm 0.038 0.379 \pm 0.027
	50 females	1.27–3.40	2.160 \pm 0.070 0.491 \pm 0.050		

TABLE—*contd.*

Item	Number of cases	RESULTS :		Comparative standards
		Range, mg. N.	Mean, mg. N.	
2. Non-protein nitrogen fraction :				
(a) Total N.P.N.	100 males	21·0-34·16	28·3 \pm 0·403 4·013 \pm 0·285	25-35 (Hawk)
	50 females	21·8-34·1	27·18 \pm 0·558 3·866 \pm 0·391	
(b) Urea ...	100 males	10·2-19·1	14·07 \pm 0·195 1·935 \pm 0·137	10-15 (Hawk)
	50 females	9·3-17·2	12·03 \pm 0·285 1·995 \pm 0·202	...
(c) Uric acid ...	100 males	2·0- 4·2	2·77 \pm 0·014 0·137 \pm 0·010	2·3-5 (Hawk)
	50 females	2·1- 3·2	2·810 \pm 0·025 0·246 \pm 0·025	...
(d) Creatin and creatinine ...	100 males	1·6- 5·4	4·590 \pm 0·017 0·166 \pm 0·012	3-7 (Hawk)
	50 females	4·2- 5·0	4·590 \pm 0·042 0·292 \pm 0·020	...
(e) Undetermined nitrogen (by calculation)	100 males	0·76-14·6	6·830 \pm 0·120 1·195 \pm 0·085	10·0 (Hawk)
	50 females	0·04-14·4	7·350 \pm 0·506 3·543 \pm 0·358	...
3. Lipid fractions :				
(a) (i) Total whole-blood cholesterol	40 males	116·1-252·4	176·4 \pm 6·101 38·098 \pm 4·259	150-250 (Wright)
(ii) Cholesterol fractions in plasma				
Total cholesterol	30 males	155·2-190·2	171·8 \pm 1·529 8·236 \pm 1·081	150-260 (Thannhauser)
Free cholesterol	30 males	47·1-65·2	54·03 \pm 0·844 4·544 \pm 0·597	40-70 (Thannhauser)
Ester cholesterol (by calculation)	30 males	99·0-143·1	117·8 \pm 1·728 9·299 \pm 1·221	110-190 (Thannhauser)

TABLE—*contd.*

Item	Number of cases	RESULTS :		Comparative standards
		Range, mg. N.	Mean, mg. N.	
3. (b) Total fatty acids ...	40 males	262-390	293.15 ± 4.764 29.755 ± 3.369	250-390 (Hawk)
(c) Lipid phosphorus in plasma ...	20 males	9.0-12.0	10.81 ± 0.189 0.823 ± 0.133	9-10 (Hawk)
4. Fasting blood sugar ...	100 males	70.7-107.5	80.290 ± 0.866 8.613 ± 0.612	75-105 (Hawk)
	50 females	70.8-107.5	82.83 ± 1.397 9.776 ± 0.988	
5. Serum calcium	30 males	9.1-11.2	10.210 ± 0.076 0.408 ± 0.054	9-11 (Hawk)
	25 females	9.3-11.2	10.24 ± 0.084 0.490 ± 0.059	...
6. Alkali reserve	23 males	34.7-48.6	42.930 ± 1.136 5.328 ± 0.803	53.77 (Beaumont & Dodds)
7. Urea clearance :				
(a) Standard	25 males		52.4 per 1.73 sq. mt.	54.0 (Bodansky)
(b) Maximum	25 males		72.0 per 1.73 sq. mt.	75.0 (Bodansky)
8. Uric acid clearance :				
(a) Standard	25 males	13.4-24.6	17.730 ± 0.635 3.110 ± 0.449	...
(b) Maximum	25 males	25.4-40.4	32.790 ± 0.750 3.674 ± 0.530	...

METHODS.

1. For plasma-protein fractions, Kjeldahl method-*cum*-titration was used in preference to other recommended methods. For prothrombin clotting time, Quick's method as modified by Magath has been used, Russel's viper venom being the thromboplastic material.

2. *N.P.N. and fraction.*—Micro-Kjeldahl involving steam-distillation was used for nitrogen determination of the protein-free filtrate obtained by Folin-Wu method. Blood urea was estimated by method given by

van Slyke and Cullen, and blood uric acid by Benedict's method. Combined creatin and creatinine was estimated by the colorimetric method after treatment with alkaline picrate. Creatin was converted to creatinine by heating protein-free filtrate with acid (Folin-Wu).

3. Total blood cholesterol was estimated by the method of Myers-Wardell as modified by Reinhold and Shiels. The cholesterol fractions in plasma were determined by the method of Schonheimer and Sperry as modified by Hawk. The total fatty acids in blood was determined by the method of Stoddard and Drury. For lipid phosphorus, the method of Youngburg was used, making use of stannous-chloride reagent of Kuttner and Cohen.

4. Blood sugar was estimated by Hagedorn Jensen method.

5. Serum calcium was estimated according to Kramer-Tisdall method as modified by Clark-Collip.

6. Alkali reserve was estimated as recommended by van Slyke and Cullen.

7. Urea-clearance values were done by the method of Moller McIntosh and van Slyke, and both blood and urine urea by use of urease method followed by æration.

CONCLUSIONS.

1. Protein fractions of blood.

Total proteins and albumin values are lower though fibrinogen values are higher. Prothrombin clotting time is double the Quick's figure.

2. The non-protein nitrogen fractions gave values which are almost normal though the protein intake in Orissa is definitely less (40 g. per day).

3. Lipid fractions.

Total blood cholesterol value of 176.4 is a normal value as well as total plasma cholesterol and its free and ester forms, total fatty acids and lipid phosphorus. This is also remarkable when the lipid intake in Orissa has been found to be as low as 11 g. to 12 g. per day.

4. Fasting blood-sugar level had a normal value.

5. Serum-calcium value was also found to be normal.

6. The alkali-reserve value of 42.93 is demonstrative of a mild type of acidosis in normal subjects.

7. Urea clearance values both maximum and standard, if the surface area is taken into consideration, was normal and not low as previously reported by some Indian workers.

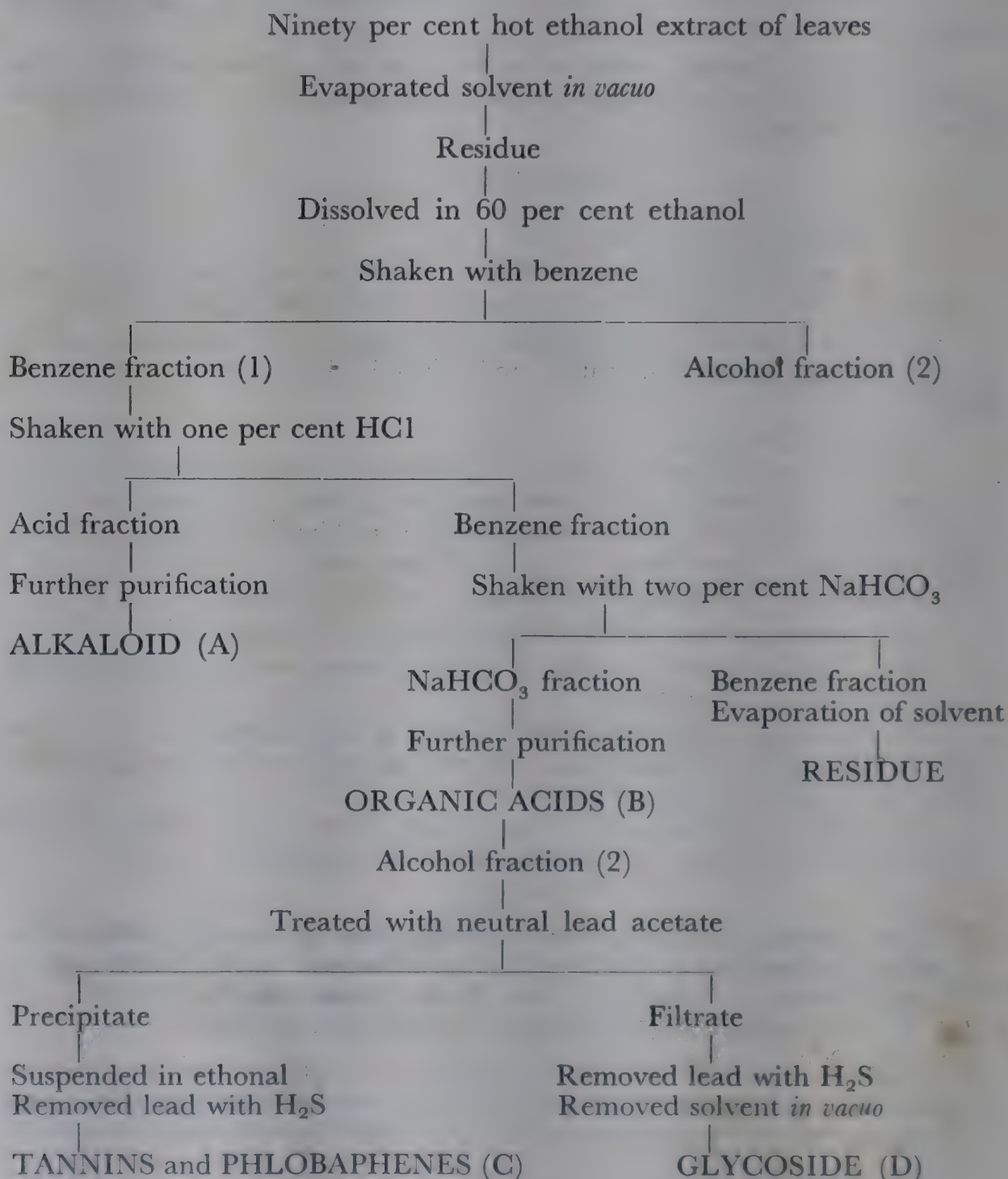
8. Uric-acid clearance values are not given in the literature, and they were found to be 32.79 (max.) and 17.73 (standard).

2. Investigation into the curative properties of certain indigenous plants belonging to the *N. O. Malvaceæ* and *N. O. Rubiaceæ* of Diabetes Mellitus by Dr. M. R. Rajarama Rao at the Central Drug Research Institute, Lucknow.

Investigations on two plants having a curative effect on *Diabetes mellitus* were undertaken after having obtained considerable proof of their efficacy by clinical trial for two years. A milk extract made from fresh

leaves of *Rivea* (Hooker flora of British India, **1**, p. 345) (*Argyriaea*) *cuneata* and the fresh bark of *Thespesia populnea* administered orally twice daily on a salt-free diet for three to five days relieved diabetic patients of the signs and symptoms of the disease. The relief was permanent in 30 per cent of the cases and temporary in another 30 per cent. Blood and urine sugar were tested in these cases at frequent intervals over a period of two years. If the sugar in urine disappeared, and in blood returned to a normal level of 80 mg. to 120 mg./100 c.c., and if the symptoms also abated, the patient was considered 'cured'. Sixteen cases were treated. This group included patients of both sexes and different age groups. The cured patients were allowed normal diet, while those who showed temporary benefit or no response at all were allowed to return to insulin and diet regimen.

The following steps indicate the method adopted for isolating the active principles from the dried leaves of *Rivea* (*Argyriaea*) *cuneata* (Hooker flora of British India, **4**, p. 191) *N. O. Convolvulaceae*.



All the four fractions were obtained in an impure state and used as such for animal experiments.

Alkaloid.—Yield of uncrystallized, but fairly pure material is 0.001 per cent. The yield is raised to 0.1 per cent if the leaves are treated with cold ammoniacal alcohol for extraction. Organic-acids yield was low and the material was not tested.

Tannins and phlobaphenes.—Yield three to four per cent of dark-brown hygroscopic powder, partly soluble in water and wholly in ethanol or methanol, and gave a green precipitate with ferric chloride. The water-insoluble fraction dissolved in a solution of two per cent NaHCO_3 .

Glycoside.—Yield of impure material 0.3 per cent hygroscopic, water- and methanol-soluble and contained free glucose and sodium chloride as impurities. A solution of this material in 80 per cent acetone or butanol saturated with water on passing through a column of activated aluminae yielded in the first fraction of the elute a pure glucose-free glycoside which on hydrolysis with one per cent H_2SO_4 on a water-bath yielded glucose and anaglucone soluble in two per cent NaHCO_3 or ether, apparently a carboxylic acid. The glycoside gave a Turnbull's blue colour with a mixture of potassium ferri cyanide and ferric chloride but no colour with ferric chloride alone. Only the impure glycoside was used in animal and clinical trials. The above fractions obtained in an impure state were used for animal experiments.

None of the fractions had any appreciable effect on the fasting blood-sugar level of normal rabbits. They were administered orally, once daily for 20 to 30 days, to alloxan diabetic rats. The glycoside (5 mg. daily) brought the blood- and urine-sugar of diabetic rats to normal after 20 to 30 days. If the treatment was stopped the rats continued in this healthy state for 30 to 40 days after which they showed signs of relapse. The phlobaphenes showed curative effect in a few diabetic rats only. The alkaloid had no curative effect.

Histological examination of the pancreas of the alloxan-diabetic rats cured by glycoside showed the presence of a few healthy islets, and in some of these the islets tissue appeared to be arising from centro-acinar cells. The pancreas of normal rats treated with glycoside for 30 days showed in some places hyperplasia of islets peri-insular sclerosis and increase in vascularization of the islet tissue.

Pharmacological experiments on dogs anæsthetized with seconal sodium showed that the alkaloid produced a rise of blood pressure and diminution of respiration; glycoside produced a rise followed by fall in blood pressure and an arrest and relaxation of intestinal movements. Glycoside had no toxic effects on mice and rats.

The pure glycoside was given to nine diabetic patients. The patients were confirmed diabetics and were under insulin and diet regimen, and their blood- and urine-sugar was tested before and after treatment, and for over two years at frequent intervals. Glycoside was given as a water solution two to three times a day for 10 to 20 days. The dosage was approximately 0.25 mg., thrice daily, by oral route. Two severe cases were given insulin, while others were maintained on regulated diet only. Four cases were cured. Their blood sugar was restored to normal at the end of the treatment, and urine sugar had disappeared. Other symptoms also abated. Treatment was stopped and normal diet was resumed.

Frequent examination of urine- and blood-sugar for over two years showed no relapse of symptoms.

The bark of *Thespesia populnea*² (Hooker flora of British India, 1, p. 345) (*N.O. Malvaceæ*) yielded four per cent of a garnet-red resin which had no curative effect on alloxan-diabetic rats.

3. Observation on the significance of malnutrition in the development of pre-eclamptic toxæmia by Dr. C. S. Dawn at the Medical College, Calcutta.

Pre-natal diet survey and nutritional assessment of 280 primigravid Bengalee women during last trimester were made during the period of 2nd May, 1951 to 1st May, 1953. Special study was made on the significance of malnutrition on the development of pre-eclamptic toxæmia of pregnancy. The sample of survey was unselected but the women were free from any organic disease other than that for malnutrition. The diet survey showed that 86.2 per cent of pregnant women lived on inadequate diet of which in 20.2 per cent the diet was grossly poor in both quantity and quality. The average daily intakes of different food factors were calories 14×76 , protein 45 g., carbohydrate 278 g., fat 21 g., iron 15.0 mg., calcium 0.35 g., phosphorus 0.7 g., vitamin A 1,007 I.U., vitamin B₁ 1.07 mg., vitamin B₂ 0.7 mg., niacin 13 mg. and vitamin C 27 mg.

Superior diet was consumed by 13.8 per cent of the women, fair diet by 66 per cent and poor diet by 20.2 per cent. 90.6 per cent of the women consuming superior diet maintained good nutrition, while those with inferior diet had fair to very poor nutrition in 95.8 per cent cases.

The average height was estimated at 58.2 ± 1.9 inches. The average weight increase during later weeks of pregnancy was 0.25 kilo per week, which figured to be the lowest as observed by different observers so far.

In normal non-toxæmic women, the systolic and diastolic blood pressures began to rise from $116.25 \pm 8.6/71.24 \pm 8.04$ mm. of Hg at 28 weeks to $117.85 \pm 9.04/74.54 \pm 9.29$ at 40 weeks. The study of both systolic and diastolic pressures and their standard deviations points to the adoption of 140/90 mm. of Hg as the critical level for diagnosing pre-eclampsia. The effect of the food factors on blood pressure was joint in nature instead of any singular effect of any particular food factor.

The average hæmoglobin value was 10.08 ± 1.26 g. per cent. The food-iron intake did not show any linear relationship with the hæmoglobin value ; but there could be found a significant effect on it by different grades of diet.

A significant correlation in the percentage incidence could be found between different grades of diet and glossitis as well as bleeding gums ; 66 per cent and 44 per cent of women developed these signs, respectively ; but no significant correlation could be found between carious teeth and calcium intake by the women.

The various grades of diet did not show any significant effect on the serum-protein and calcium levels. The former varied from 5 g. to

6.5 g. per cent, while the later showed the values between 8 mg. to 10.5 mg. per cent. Estimations of plasma riboflavin showed the average value of 0.32 μ g. per cent.

There could be found an appreciable association between inferior diet and the development of a high incidence of pre-eclampsia. Of the deficient food factors in the inferior diet, poor intakes of calories, proteins, calcium, iron, vitamin A, vitamin B complex (especially riboflavin) and vitamin C were found to have a significant effect when they acted jointly ; but none of them acting singly caused any appreciable effect on the development of the disease.

Supplementary feeding experiment by Vitamin B complex showed some effect in lowering the incidence of the disease and such effect was statistically significant.

4. Rheumatoid arthritis and its response to TAB by Dr. K. K. Sikka at the K. G. Medical College, Lucknow.

Rheumatoid arthritis is one of the most common cause of chronic illness at the present time, and disables each year many individuals. Since the thrilling discovery of ACTH and cortisone, cases have been reported in which complete remission has been noticed particularly if the disease is in early stage. It is, therefore, necessary to study the clinical aspect of the disease to diagnose it at an early stage and to revive the well-recognized good old remedy of pyreto-therapy which is supposed to have a beneficial effect in the diseases through the liberation of ACTH in the system. An attempt has also been made to study the ætiology of the disease because various factors have been arraigned as causing or pre-disposing to rheumatoid arthritis.

The present work is based on careful observation on 100 cases admitted to Gandhi Memorial and Associated Hospitals and Balrampur Hospital from April 1950 to April 1953.

The detailed histories of the cases have been recorded according to the investigations laid down by the Scientific Advisory Committee of Empire Rheumatism Council of England (*Brit. Med. Jour.*, 8th April, 1950).

The classification of the clinical variants of the cases have been done according to the classification introduced by the Scientific Committee of the Royal College of Physicians (1934).

To assess the value of pyreto-therapy in the treatment of rheumatoid arthritis, therapeutic criteria have been adopted according to the Committee for Therapeutic Criteria of New York Rheumatism Association, as they are entirely based on objective signs. (*Jour. Amer. Med. Assoc.*, 25th June, 1949).

Special biochemical tests, such as 17-ketosteroids excretion in the urine and urinary uric-acid: creatinine ratio, have been done in 35 cases in order to confirm the probable mechanism of action of pyreto-therapy. The methods employed in each case and the difficulties encountered in standardizing the technique of each test have been fully described. The estimation has, however, been carried out by the methods of Robin and Gibson (1934) and Callow *et al.* (1938) with the exception that in the final development of colour by Zimmerman reaction, the method of Holtroff

and Koch was adopted and the Klettsummerson photo-electric colorimeter was standardized. Excretions of 17-ketosteroids in normal subjects of both sexes have been done in 65 cases. In addition to this, other laboratory tests were performed for differential diagnosis.

During this period ACTH was administered to a resistant case of rheumatoid arthritis. He was able to procure a limited quantity of the drug from the Armour Laboratories, Chicago.

In the following paragraphs is enlisted some impressions which appear to be most vivid and striking regarding the ætiology, onset, clinical pattern and treatment with pyreto-therapy :—

A. ÆTIOLOGY.

The significant points are :—

1. *Age incidence*.—The mean age for males is 27·9 years, and for females 32·9 years. These figures seem to support the view commonly expressed in the literature that rheumatoid arthritis has a special pre-dilection for young individuals.

2. *Sex incidence*.—Males predominate both in case incidence as well as familial collecteral incidence.

(a) Case incidence ... Males to females : 60 cases to 40 cases.

3. *Caste*.—There is no special incidence in particular caste. Hindus constitute 76 per cent (76 cases) of the total 100 cases.

4. *Occupation*.—Seventy per cent were mannual workers, 60 per cent assumed fixed sitting posture, 78 per cent stated that they were working in damp surroundings and 34 per cent presented with postural deformity. Posture at work (sitting 60 per cent as compared to standing 30 per cent) appears to be more important than the nature of work. Damp surroundings seem to pre-dispose the individual to rheumatoid arthritis.

5. *Diet*.—Seventy-six per cent were vegetarians, 22 per cent were non-vegetarians. Diet was inferior in quality in most of the cases. Sixty per cent with a clear dietatic history were consuming excessive salt in the diet. Quality of diet (especially low in first class proteins and excessive salt in the diet) appears to pre-dispose an individual to arthritis.

6. *Focal infection*.—This was present in 44 per cent of cases in the present series. It appears to play some part in the ætiology of the disease and should be considered as a trigger helping to upset the endocrine metabolic balance of the body.

7. *Family history*.—It was forthcoming in 54 per cent of the cases, stressing the importance of some environmental conditions and quality of diet to which all the members of the family are equally exposed.

8. *Past illness*.—History of past illness was forthcoming in 82 per cent of cases. There is some evidence that some cases of rheumatoid arthritis may be due to an abnormal response on the part of the patient to an antigen, a few may result from venereal diseases, while majority may have suffered from some infectious diseases in the past prior to onset of arthritis,

B. ONSET.

Significant points are :—

1. *Psychological stress*.—This, in its many forms, is capable of precipitating or exaggerating an already existing disease. Sixty per cent of the cases in the present series within the preceding two years had some important incidence leading to depression and dejection.

2. *Relation to pregnancy, menopause and menstrual cycle*:—

(a) The fact that pregnancy brings about spontaneous remission was confirmed and none showed any casual relation to pregnancy.

(b) Menstrual irregularities and low fertility (63·1 per cent) were frequently associated prior to the onset of the disease.

(c) Menopause seemed to have less ætiological significance.

3. *Home conditions*.—Dampness appears to pre-dispose the individual to rheumatoid arthritis. Seventy-eight per cent of cases were living in damp houses.

4. *Peripheral vascular abnormalities*.—The clinical impression of the frequency with which the peripheral circulatory changes are found in rheumatoid patients is confirmed as they were present in 80 per cent of cases and that they anti-dated the onset.

5. *Type on onset*.—Onset is more often insidious (75 per cent) than acute and in 40 per cent it was febrile in onset.

C. CLINICAL PATTERN.

Significant points are :—

1. Rheumatoid arthritis is definitely a systemic disease involving many tissues and organs on account of the numerous prodromal symptoms pertaining to different organs are complained by the patient.

2. Peripheral joints are more often involved constituting 67 per cent in this series but the onset may be from proximal joints to begin with (30 per cent in this series).

3. All the clinical features of the disease have been largely confirmed except that the incidence of ulnar deviation was much less, constituting only 20 per cent in the present series.

4. Relation of weather (positive in 51 out of 60 cases) and season (positive in 28 out of 40 cases) to the disease is in keeping with the widely accepted belief that rheumatoid arthritis is usually influenced adversely by cold and damp conditions.

D. LABORATORY FINDINGS.

1. *17 ketosteroids in urine*—

(a) Normal subjects—done in 65 cases of all ages.

Mean values in males (Indian) 14·1 mg. in 24 hours.

(Range : 11·5 to 16·8)

Mean values in females (Indian) 11·05 mg. in 24 hours.

(Range : 8·7 to 11·8).

(b) *Rheumatoid patients*.—Mean values for males 11.5 mg. in 24 hours.
Mean value for females 8.1 mg. in 24 hours.

On the whole 17-ketosteroids excretions were low in 61.7 per cent, within normal limits in 23.4 per cent, while in 14.7 per cent it was in higher normal range. The excretion of 17-ketosteroids in rheumatoid patients shows a progressive decline with the rheumatoid activity and with the duration of illness.

2. *Other laboratory findings*.—ESR is closely related with rheumatoid activity and increases with the stage of the disease. Eosinophils also show a progressive increase with the rheumatoid activity.

3. *No anæmia* could be demonstrated in 32 per cent. Fifty-two per cent microcytic anæmia and ten per cent were in macrocytic group.

4. *Serum proteins*.—Total serum proteins usually remain normal throughout the illness and it is only the globulin fraction which increases with the rheumatoid activity and duration of illness.

	G. per cent
Mean value of total serum proteins	7.1
Mean value of albumin	3.1
Mean value of globulin	4.0

5. *Blood-uric acid*.—This was found to be in the normal range in all cases of rheumatoid arthritis, whereas x-ray findings were positive in in 79 per cent of the cases.

E. TREATMENT.

Altogether 100 patients received artificially produced pyrexia.

<i>Response</i> .—	Per cent
<i>Grade I</i> —(complete remission)	54
<i>Grade II</i> —(major improvement)	20
<i>Grade III</i> —(minor improvement)	16
<i>Grade IV</i> —(unimprovement)	6
Spontaneous remission	2
Death (due to peritonitis)	2

In conclusion, it may be said that pyreto-therapy had an important place in the treatment of rheumatoid arthritis and other disorders where no specific treatment was available.

The beneficial effect of pyreto-therapy is brought about through pituitary-adrenal axis excess leading to increased gluco-corticoid activity. This fact is proved by the increase in the urinary uric-acid: creatinine ratio and complete disappearance of eosinophils from peripheral circulation and increased excretion of 17-ketosteroids after the pyrexia which remains above the basic level for two to three days.

This form of treatment is contra-indicated in any patient who would not be considered a good risk for a major operation.

Follow up.—

Twenty-four per cent had no pain for one year after the treatment, 20 per cent had no complaint for six months, and 16 per cent reported to

have no re-currence of symptoms within two months after the expiry of treatment. Condition became worse in eight per cent, while it remained stationary in two per cent of the cases.

5. Tropical eosinophilia by Dr. Sami Hameed at the K. G. Medical College, Lucknow.

Clinical investigations of 100 cases show that hepatomegaly, splenomegaly and adenopathy could not conceivably be found associated with the bronchitic and asthmatic symptoms of tropical eosinophilia. Two cases were observed who had massive eosinophilia without pulmonary symptoms and signs, but instead presented symptoms of general ill-health. These cases responded to arseno-therapy and, therefore, the name 'tropical pulmonary-eosinophilia' of Crofton and Bell (1950) does not seem to be appropriate and hence a new nomenclature 'tropical eosinophilæmia' is suggested. Sternal bone biopsies indicate that eosinophilæmia in these cases is due to intra-medullary proliferation of eosinophil precursors. Studies on arseno-therapy indicate that there is exaggeration of symptoms and elevation of blood eosinophils after the first dose of arsenic, which is followed by a fall in subsequent injections. Experimental investigations show that bacteria-free biologic-fluid of tropical eosinophilia patients produce rise of eosinophils in the inoculated animals, as compared to control animals. Moreover, the bacteria free sputa and sera of patients are capable of agglutinating chicken r.b.c. An indirect indication of virus is being involved in the process. Electron-microscopic studies also show the presence of particles in the patient's sera, whether these particles have any thing to do with tropical eosinophilia cannot be said at this stage, but rise of eosinophils in animals inoculated with bacteria-free sputa and sera, the ability of the patient's sera and sputa to agglutinate chicken r.b.c., the presence of particles which are smaller than the smallest bacteria in patient's sera on electron-microscopic studies, and the repeated failure to isolate any other organism by the author of the thesis and other later-dated workers, all go to suggest that tropical eosinophilia is of virus ætiology.

6. Electrolyte balance in health and diseases by Dr. S. K. Vaish at the Indian Veterinary Research Institute, Izatnagar.

Movements of the fluids and electrolytes within the organism and the mechanism involved in the exchange of these substances with the external environment are of interest to all concerned because a knowledge of their abnormalities is essential for the treatment of many diseased conditions.

The work has been carried out on the following lines :—

1. Effect of different levels of carotene on the metabolism of electrolytes.
2. Effect of vitamin-A deficiency on the concentration of electrolytes in blood and tissues.
3. Variations in the concentrations of serum electrolytes in diabetic acidosis and coma.
4. Therapeutic use of Jaman seed in *Diabetes mellitus*.
5. Physiological studies in human beings.

1. *Effect of different levels of carotene in the metabolism of electrolytes.*—Adult male and female rats maintained on vitamin-A deficient diet were mated. The litters obtained were weaned when the average bodyweight was 30 g. Four groups of four litters each having almost the same average weight were selected for experiment.

All the four groups were fed vitamin-A deficiency diet supplemented with 16 τ , α -tocopherol and 20 I.U. of calciferol per rat per day till the average body-weight was about 100 g. The diet was then supplemented with 0.5 τ , β -carotene, 2.0 τ , β -carotene and 10.0 τ , β -carotene, respectively, for the 2nd, 3rd and 4th groups. The first group was kept as controls. The feeding of the supplemented diet for seven days was followed by a collection period of four days.

The basal ration, and the faeces and urine collected during the metabolic period were analysed for Ca, P, Mg, Cl, Na and K. It was observed that with the gradual increase in the dosage of β -carotene, within the experimental range, the absorption of Ca, Mg, P and Cl was improved, whereas in the case of Na and K a depression in the absorption was noticed. The above data were also put to statistical examination. The 't' values in the case of Mg, Cl, Na, and K were found to be significant.

2. *Effect of vitamin-A deficiency on the concentration of electrolytes in blood and tissues.*—The examination of blood of rats fed on stock and vitamin-A deficient diets showed significant differences in the concentration of chlorine, sodium and potassium. Although the differences in the cases of these electrolytes were significant, the values lay within normal limits for chlorine and sodium, whereas in the case of potassium, the concentration in blood of the vitamin-A deficient group of animals rose to higher than normal range, being 36.5 ± 1.32 mg./100 c.c. of blood serum in rats fed on vitamin-A deficient diet as compared to 24.92 ± 1.67 in the case of rats fed on stock diet.

The tissues analysed were spleen, heart, kidney, liver and muscles. The concentration of calcium, magnesium and phosphorus was depressed in tissues of vitamin-A deficient rats as compared with the tissues of rats of control group, whereas no definite trend of either decrease or increase in the concentration of chlorine, sodium and potassium was observed.

The differences in concentration of electrolytes in tissues of rats fed on vitamin-A deficient diet and stock diet were, however, significant for calcium in heart and kidneys, for magnesium in spleen and heart, for phosphorus in kidneys and liver, and for sodium and potassium in heart only. The differences in the concentration of chlorine in the experimental and control groups were found to be not significant in any of the tissues analysed.

3. *Variations in the concentrations of serum electrolytes in diabetic acidosis and coma.*—In view of the fragmentary evidence available on the concentration of blood electrolytes in diabetic acidosis, a systematic study was undertaken to determine the Ca, P, K, Cl, Na, Mg and proteins values.

For these experiments dogs were selected as experimental animals. Since physiological values for the concentration of these electrolytes in blood and serum of dogs under Indian conditions are not available, the experimental study was preceded by the determination of normal values in 12 healthy dogs.

In order to find if diabetic acidosis causes any variation in the electrolyte make-up of blood, nine healthy dogs were selected. Three of them were kept as controls and the other six formed the experimental group. Artificial diabetes was produced in the experimental group. Glucose-tolerance test was performed both in the control and experimental animals. The bicarbonate concentration was found to be 0.687 ± 0.095 ml. of 0.01 M NaHCO_3 per ml. of plasma in the experimental animals compared to 1.127 ± 0.051 c.c. in control. The corresponding pH values for the experimental and control animals were found to be 7.46 ± 0.042 and 7.23 ± 0.061 , respectively. Both inorganic phosphates and K were increased to 4.02 ± 0.541 and 7.76 ± 0.653 mEq/lit., respectively, in the experimental animals as compared to 2.07 ± 0.15 and 5.15 ± 0.378 in the control group.

Observations analogous to the above also carried out in dogs in which diabetic coma was produced experimentally. In diabetic coma the changes in the concentration of electrolytes in blood were more marked than in diabetic acidosis. Here again, the bicarbonate concentration was found to be 1.16 ± 0.09 ml. and 0.28 ± 0.08 ml. of 0.01 M NaHCO_3 per c.c. of plasma in the control and experimental animals, respectively. The corresponding values for the pH were 6.99 ± 0.078 and 7.44 ± 0.07 in the experimental and control animals, respectively. Inorganic phosphorus and potassium were 6.72 ± 0.084 and 10.515 ± 1.412 mEq/lit., respectively. In addition chloride concentration was 88.55 ± 4.68 mEq/lit. in the experimental animals as compared to 100.17 ± 4.598 in the controls. Protein concentration was raised from 18.23 ± 0.87 in the controls to 22.07 ± 0.97 mEq/lit. in the experimental animals.

4. *Therapeutic use of Jaman seed in Diabetes mellitus.*—Twelve healthy rabbits of the same colony of approximately the same age and weight were selected. They were divided into four groups of three each, so that the average initial blood-sugar level was the same in each group. The animals were made diabetic by intravenous injection of ten per cent solution of alloxen monohydrate (B.D.H.) in pyrogen-free distilled water in the dosage of 200 mg./kg. body-weight of the animal.

One group was kept as control, and powdered fresh Jaman-seed was offered in the dosages of 0.75 g., 1.00 g. and 1.25 g. per diabetic animal in the 2nd, 3rd and 4th groups, respectively. The ingestion of Jaman seed was observed to lower the level of sugar in blood which became constant at about 130 mg./100 c.c. (normal value 70-100 mg./100 c.c.) after nearly five weeks. When no further lowering was obtained in the blood-sugar level the injection of Jaman seed was stopped after seven weeks.

The blood-sugar level remained stationary for about four to five weeks and then showed a tendency to rise though even after 18 weeks the original level has not been reached.

At this stage, Jaman-seed feeding was again started just as before to all the experimental group of animals, i.e. 2nd, 3rd and 4th groups. The blood sugar again began to fall after Jaman-seed feeding has started.

It seems that Jaman-seed powder has some effect in reducing the blood-sugar level in either of the following two ways :—

1. It helps in the regeneration of the islets of Langerhans.

2. The blood sugar remains low as long the cumulative effect of the active principle of the Jaman-seed in the tissue lasts.

We hope further experiments will clear the point in question.

5. *Physiological studies in human beings.*—Since food is one of the most important factors which influences body fluids and its components, it was considered desirable to find the concentration of electrolytes and total proteins in blood as affected by north Indian diet. Blood of twelve healthy males of approximately the same age fed on north Indian diet was analysed for calcium, phosphorus, magnesium, chlorine, sodium, potassium and total proteins.

The average concentration in blood was found to be 10.30 ± 1.29 mg./100 c.c. of blood for calcium, 4.03 ± 1.096 for phosphorus, 1.99 ± 0.28 for magnesium, 325.00 ± 41.94 for chlorine, 363.98 ± 44.36 for sodium and 22.28 ± 3.47 for potassium. Blood proteins varied from 6.19 to 8.00 mg./100 c.c. of blood with an average of 7.26 ± 0.663 .

7. Biochemical and clinical investigation of nutrition œdema by Dr. K. L. Mukhopadhyia at the School of Tropical Medicine, Calcutta.

The clinical observations were made on 83 patients admitted into the hospital. They were mostly very poor; it was, however, striking that the other members of the families of the patients who used to live under the same conditions usually did not have any complaints. Malnutrition as such was, therefore, not sufficient to give rise to the clinical symptoms, other conditioning factor being required to develop the disease. This is possibly some concomitant bowel disorder.

The usual complaints on admission, besides, œdema were alimentary troubles, such as indigestion, diarrhœa, poor appetite, followed by loss of weight and weakness. The diets consumed by the patients even when they were not ill, were poor in calories, more so in protein. The advent of diarrhœa necessitated a further reduction in diet resulting in a negative balance which culminated in the loss of weight, progressive weakness and finally in the œdema of the body. The œdema was usually confined to the lower extremities, but was generalized in some cases with ascites and hydrothorax. The onset of the œdema was insidious. The mild cases lost their œdema by the first week's stay in bed when they received hospital rest and diet. The severe cases, though they lost some weight, showed little or no difference in their œdema during the first ten days or so of their stay. Some patients took as long as two months to become free of œdema even when early dietary treatment was adopted. The œdema tended to come and go in cycles in these patients. Although the œdema represented usually the most prominent symptom, the patients often showed some other characteristic signs. The skin was affected in nearly all the patients; the mild cases showed perifolliculosis and the severe ones crazy-pavement dermatosis; there was also some marked hyperkeratosis on parts exposed to friction. In four patients the hair was discoloured; there was no nail change in any. The patients were oliguric on admission. The mild cases showed diuresis on bed rest. The urinary output was greater during the night than during the day, this being a status which has also

been described in patients suffering from cirrhosis of the liver. Even when the diuresis started in the course of the stay in hospital the same reversed rhythm of the urinary output was noticeable. The stools were often pale and diarrhœic. No pathogenic organisms were found on repeated examinations of the stools in majority of the cases. Stool cultures were negative for non-lactose fermenters. On sigmoidoscopy no ulceration or inflammation of the mucous membrane was detected; in a few the mucosa appeared œdematous. Some patients showed atrophic glossitis with no marked inflammation. A few had rather severe glossitis and angular stomatitis as well. The whole of the intestinal tract appeared to be affected; usually an insufficient secretion of hydrochloric acid from the gastric mucosa was observed. No estimation of other intestinal secretions was made so far; however, such findings were reported for the closely related syndrome of Kwashiorkor of which three cases were encountered.

Two patients developed re-feeding gynæcomastia during their period of recovery. In some of the patients under observation, Bitot's spots on the conjunctivæ were strikingly prominent.

There was marked hypo-albuminæmia in all the cases. The A : G ratio was altered. No correlation between the level of serum proteins and the degree of œdema could be found. The onset of diuresis and diminution of the œdema did not coincide with any increase of serum proteins; as a matter of fact, there was a considerable lag between the disappearance of œdema and return of the serum proteins to normal. Serum cholesterol and chlorides were on the lower side of normal; however, no statistically significant difference could be detected. Fasting blood-sugar level was usually on the lower side of normal. Only in three patients a marked hypoglycæmia was found. The oral glucose-tolerance test was not infrequently flat suggesting impaired absorption. The intravenous glucose tolerance in the cases showing flat curves was found to be normal. Bilirubin content of serum was usually within normal range or slightly raised, while the serum cholesterol was on the low side. Thymol-turbidity tests showed high values in some patients and in the rest it was normal. Liver biopsy done in two cases showed fatty infiltration in the liver parenchyma and increased fibrosis at the periportal regions in one case and degeneration of hepatic cells in another. There was evidence of increased fat excretion with the stools in this condition.

The plasma volume per kg. of body-weight in the patients whose œdema was increasing was found to be reduced as compared with the normal. The plasma volume increased with the improvement of the clinical state. The thiocyanate space per kg. of body-weight was increased almost to double of its normal value when they were œdematous; it gradually declined, and even when they were clinically free of œdema was still higher than normal. It usually took about a fortnight after the clinical disappearance of the œdema for the thiocyanate space to return to normal. It, therefore, appears that the striking feature of the œdema observed in patients, suffering from nutritional deficiency, is the considerable expansion of the thiocyanate space even up to double of the normal average, which is not accompanied by an adequate increase of the circulating plasma volume. On the contrary, the plasma volume is reduced as compared to its normal value. There is considerable delay in the excretion of the test dose of water in patients suffering from nutritional œdema.

It appears probable that the reduction of plasma volume leading to a diminished glomerular filtration rate and glomerulotubular imbalance, together with the hypoproteinæmia with its altered renal hæmodynamics is responsible for the excessive re-absorption of sodium and thus gives rise to expansion of the extra-cellular space and œdema.

8. Studies on body-fluid changes in cholera by Dr. J. K. Biswas at the Nilratan Sirkar Medical College, Calcutta.

The object of the work was to study the depletion of body-fluids from extra-cellular compartments in cholera and its restoration by different transfusion fluids. This was done from estimation of blood and plasmas pecific gravities, packed cell volume, plasma volume and available thiocyanate space at different stages of restoration.

The findings with regard to the initial state of de-hydration and hæmo-concentration are summarized in Table I :—

TABLE I.

Items	Average of normal cases	Average of 66 cholera cases	Deviation
Blood specific gravity ...	1,054	1,068	+14
Plasma specific gravity ...	1,026	1,038	+12
P.C.V. ...	43	58	+15
P.V. in c.c./kg. ...	48·5 *	38·9	— 9·6
T.T.S. in c.c./kg. ...	232·9 *	189·3	—43·6

*Mean of the average of normal figures published by Chaudhury, *et al.* (1951) (*Ind. Jor. Med. Res.*, **39**, p. 553 and **39**, p. 237) and Chatterjee (1952). (*Jour. Ind. Med. Assoc.*, **21** p. 231.)

The types of transfusate for the treatment of the initial stage of de-hydration are as follows :—

	Cases
Hypertonic saline (Gr. 120 to 01), ...	16
Normal saline (Gr. 90 to 01) ...	14
Hypotonic saline (Gr. 60 to 01) ...	8
Plasmosan ...	6

The effectiveness of the solution in repairing the de-hydration has been evaluated by estimation of the specific gravity of the blood and plasma, B.P. level, the plasma volume and total thiocyanate space, before and after the transfusion. The duration of the maintenance of the restored state has also been taken into account.

In the initial stage each group was subdivided into those with a systolic B.P. of 50 mm. Hg and above, those and with B.P. below 50 mm. Hg on the basis of work by Das *et al.*

The data are summarized in Table II :—

TABLE II.

*Restoration studies after 1st transfusion.**

Type of saline	Number of cases	Quantity of saline /kg.	DEVIATION FROM NORMAL (BEFORE SALINE)				DEVIATION FROM NORMAL AFTER 1ST TRANSFUSION			
			Blood specific gravity	Plasma specific gravity	P.C.V.	P.V. in c.c./kg.	T.T.S. in c.c./kg.	Blood specific gravity	Plasma specific gravity	P.C.V.

Those with blood pressure below 50 mm. Hg—Total cases=30.

Hypertonic	12	38·8	+15	+13	+16	-12	-51·5	-1	-2	0	+7·8	-14·8
Normal	10	37·1	+14	+12	+16	-13	-46·4	+4	+2	+5	+0·4	-12·5
Hypotonic	4	36·1	+14	+14	+16	-10·3	-40·9	+6	+3	+6	+1·9	-11·6
Plasmosan	4	36	+15	+13	+15	-10·5	...	+3	+3	+2	+2	...

Those with blood pressure above 50 mm.—Total cases=30.

Hypertonic	4	35·6	+13	+12	+11	-9·3	-39·5	-1	-2	-1	+10·8	-7
Normal	4	38·7	+11	+9	+11	-8·9	-40·9	0	+1	+2	+5·7	-4·5
Hypotonic	4	35	+9	+8	+10	-5·8	-30·4	-1	0	0	+6·8	-1·6
Plasmosan	2	34	+15	+12	+16	-9·5	...	0	-1	0	+5·9	...

*Data of 4 cases who died during the first transfusion are not included in the series.

After the improvement attained by the first transfusion, some patients showed a permanent restoration, others deteriorated after the initial improvement so that the blood specific gravity gradually increased and reached at or near 1,063. At this stage a further transfusion was given, and the four different transfusates already mentioned were given to different cases, in rotation as far as possible. The analysis of the cases are given in Table III:—

TABLE III.*

Type of saline	1 Number of cases	2 Permanent improvement of initial condition	3 Number requiring further transfusions	4 Deaths
Hypertonic saline	16	6	10	One death after second transfusion.
Normal saline ...	14	5	9	
Hypotonic saline	8	1	7	Nil
Plasmosan ...	6	2	4	One death after second transfusion.

*Data of 4 cases who died during the first transfusion are not included in the series.

The detailed results of the cases in column 3 where subsequent transfusion was necessary are given in Table IV :—

TABLE IV.

Subsequent transfusion — deviations after 2nd transfusion.

	Number of cases.	Saline per kg. body-weight, c. c.	DEVIATION FROM NORMAL BEFORE TRANSFUSION.			FINAL DEVIATION FROM NORMAL AFTER 2ND TRANSFUSION.			Permanently maintained 2nd transfusion.
			Blood specific gravity.	Plasma specific gravity.	P.C.V.	Blood specific gravity.	Plasma specific gravity.	P.C.V.	
Hypertonic followed by hypertonic. ...	2	36.4	+10	+8	+13	+3	+1	+6	1
Hypertonic followed by normal. ...	4	41.0	+9	+7	+10	-1	0	-1	4
Hypertonic followed by plasmosan. ...	4	40.5	+9	+7	+10	-1	+1	0	4
Normal followed by normal. ...	9	36.0	+10	+7	+11	+1	+1	+3	6
Hypotonic followed by hypotonic. ...	7	35.5	+10	+7	+11	+2	+2	+3	4
Plasmosan followed by plasmosan. ...	4	36.3	+9	+7	+9	-1	+1	-1	1

The findings summarized in Table I to IV can be briefly commented upon in two stages:—

Stage I : The state of initial de-hydration.—There was uniform increase of the specific gravity of blood and plasma. The marked increase of the plasma specific gravity indicates an active loss of fluid from the plasma. The increase in the whole-blood specific gravity is brought about by shrinkage of plasma volume. This is also shown by the increase in P.C.V. (35 per cent above the basic value).

The percentage of loss of fluid from plasma (19 per cent) is almost equal to that of the interstitial space (18 per cent). This, however, does not indicate the absolute state of de-hydration. Considering the fact that normally the total volume of interstitial fluid was found to be 3.3 times to plasma volume, it appears that the interstitial space was depleted by 3.4 times as much water as the plasma.

Stage II : Restoration studies—Restoration of initial state of de-hydration.—Total number of cases studied was 44.

Hypertonic saline was given to 16 cases. In all cases the restoration was such that the blood and plasma specific gravity as well as P.C.V. were nearly restored to normal. The change was more clearly evident in the group of 12 cases where the B.P. was less than 50 mm. Hg.

Results attained with normal saline (14 cases) only showed a fair restoration in the seriously ill patients (ten cases), and good restoration in those (four cases) with B.P. above 50 mm. Hg. Hypotonic saline and plasmosan had almost similar results to that of normal saline.

Considering the three items, blood specific gravity, plasma specific gravity and P.C.V. restoration obtained with hypertonic saline was more satisfactory.

The immediate normalization of plasma volume and thiocyanate space appears to occur better with normal and hypotonic saline than with hypertonic saline. However, the latter gives rise to a stage of hyper-compensation with regard to the plasma volume as it expected, with slight but appreciable under-compensation of the thiocyanate space.

The greatest improvement judged clinically as well as by serial specific gravity studies, and by the duration of maintenance of the restored state (five and a half hours) occurred with hypertonic saline. The corresponding duration for the other three varieties of infusion did not show any significant difference.

With regard to the 2nd transfusion, it appears that the best restoration can be obtained when hypertonic saline is followed by normal saline. More or less similar results may be expected when plasmosan is used as the 2nd transfusate. When normal saline or hypotonic saline was repeatedly infused from the initial stage, the results with regard to normalization were not so satisfactory.

9. A scheme of biochemical research for eliciting the causes of frequent occurrence of toxæmias of latter months of pregnancy by Dr. A. P. Chakravarty at the Medical College, Calcutta.

The biochemical investigation which forms the subject of the report was initiated by estimating the plasma protein (by micro-Kjeldahl method) in both normal and toxæmic cases of same gestation period (24th to 40th weeks). The original purpose was to define a relation of low blood protein or altered albumin: globulin ratio to toxæmia of pregnancy. As the results did, not however, tally with the clinical findings, it was decided to carry the research on the more fundamental basis.

That the disease occurs only during pregnancy, although in some occasions they do occur even sometimes after confinement, suggests that either placenta or decidua or both are at fault. Therefore, it was decided to study the placental biocemistry.

The object of the work was :

1. To study the activities of the placental de-aminating enzyme under both normal and toxæmic conditions.
2. To study the prosthetic group of the placental de-aminating enzyme in normal and toxæmic conditions.

MATERIALS AND METHODS.

In all 51 placentas were included in the placental de-aminating enzymatic study. The collection of materials was restricted from normal first gravida and toxæmias of pregnancy. The purpose of such a selection was that the incidence of pregnancy toxæmia is very high among first pregnancies. Only cases of pre-eclampsia, eclampsia and recurrent toxæmias were included under the heading of toxæmias of latter half of pregnancy. The 'nephritic' and 'hypertensive' toxæmias were excluded from this study by :—

(a) Routine examination of cardiovascular system for enlargement of heart and thickening of radial artery.

(b) Urine examination for sp. gr. (values less than 1,010 rejected).

(c) Urea concentration test (values less than 2.5 per cent rejected).

(d) Microscopical examination of urine for granular or epithelial casts.

(e) Ophthalmoscopic examination to visualize directly for any organic changes in the vessels of retina (cases showing arteriosclerotic changes) were rejected.

The placentas were collected immediately after delivery and kept in refrigerator in which they remained up to three to four hours for setting up the experiment.

METHOD.

The oxidative de-aminating capacity of the placental enzymes mono-amine oxidase were studied according to the technique of Thompson and Tickur (*Biochem. Jour.*, 1949, **45**, p. 125). The substrate used was tyramine hydrochlor (supplied by L. Ligh & Co., England). The experiments were carried out in an atmosphere of pure oxygen.

The experimental results are included in the detailed report.

Studies into the prosthetic group of the placental mono-amine-oxidase system in normal and toxæmic pregnancy.

References from the literature do not give any definite idea regarding the exact nature of the prosthetic group of the enzyme mono-amine oxidase. However, p-chloromercuribenzoate depresses the mono-amine oxidase considerably, indicating that an SH group is of importance in the enzymatic degradation of pressor amines.

CONCLUSIONS.

1. The presence of an active mono-amine oxidase in normal-term placenta has been corroborated.

2. Placental mono-amine-oxidase activity in toxæmia cases is found to be consistently lower than from normal pregnancy. This altered activity cannot be accounted by fall in oxygen tension alone, as there was a sufficient difference even when the oxygen tension in the surrounding medium was 100 per cent saturated.

3. Since there was a much lower value of placental mono-amine-oxidase activity in toxæmia pregnancy even with 100 per cent oxygen saturation, it is reasonable to think that a fall in oxygen tension (e.g. through uterine ischæmia as formerly believed) alone cannot account for the possible mechanism of hypertension (which is taken as an index of toxæmia) in pregnancy toxæmia.

4. This shows that there are some other causes which primarily alters the placental mono-amine-oxidase activity in toxæmic placenta, and this allows the foetal metabolites to pass unchanged and produce its effect in the sensitized vascular system of the latter half of pregnancy.

10. Purification and chemical structure of immune proteins and active immunizing agents with the use of crystalline proteolytic enzymes by Shrimati S. S. Rao at the Haffkine Institute, Bombay.

This work was undertaken to study the action of crystalline proteolytic enzymes on bacterial toxins and toxoids and secondly to elucidate the chemical structure of these biologically important substances. This is possible because different proteolytic enzymes, such as trypsin, chymotrypsin, pepsin, papain and carboxypeptidase act on different linkages in the proteins. Apart from the theoretical results expected in this investigation, it was thought possible that some of the results might indicate a better method of purification of the protein antigens, such that they may be more useful for immunization and be free from concomitant impurities which often cause severe reactions.

The action of crystalline trypsin and chymotrypsin on diphtheria and tetanus toxins and toxoids has shown that toxins are easily digested by crystalline trypsin, whereas the toxoids are very resistant. Action of crystalline trypsin on diphtheria toxin and toxoid was studied in great detail. The studies revealed that in the process of de-toxification the guanidine group of arginine and the E-amino group of lysine are bound by formalin. This has been partly proved by making use of the paper-partition chromatography to support our assumption. Besides this, there may also be steric re-arrangements brought about by the action of formalin on diphtheria toxin. The resistance developed to heating may be due to methylene-bridge formation between a terminal amino group and any other functional group, free or bound. During incubation with formalin diphtheria toxin loses toxicity more rapidly but the resistance to heat denaturation and to tryptic digestion is developed slowly and they run parallel.

This study has helped in the purification of toxoids. It was found that the impurities in crude toxoids are digested away by proteolytic enzymes leaving the specific toxoid intact. Action of very pure pepsin on diphtheria toxin and toxoid showed that diphtheria toxin is more resistant to pepsin than to either trypsin or chymotrypsin. Very conclusive results could not be obtained as diphtheria toxin is very unstable at the pH which is optimum for peptic digestion.

The study of action of crystalline trypsin, crystalline chymotrypsin, and very pure pepsin, on crystalline egg albumin showed that crystalline trypsin does not digest native egg albumin, whereas it rapidly digested heat-de-natured egg albumin. Crystalline trypsin failed to digest acid-de-natured egg albumin and so also there was not marked digestion of urea de-natured egg albumin. Formalinized-egg albumin was resistant to heat coagulation — a property which is also shown by formalinized diphtheria toxin. In addition, egg albumin showed that during incubation with formalin the tyrosine groups were bound as shown by its failure to develop colour with phenol reagent.

11. Isolation of new antibiotics from soil by Shrimati S. Chandra Sekhar at the Indian Agricultural Research Institute, New Delhi.

Different media were inoculated with dilutions of soils to isolate organisms producing antagonistic substances against *S. aureus*, *B. subtilis* and *B. coli*.

Twenty-eight actinomycetes were found active against *S. aureus* and *B. subtilis* but not against *B. coli*.

These were grown on liquid media containing eight different nitrogen sources namely, soya-bean meal, trypsin, glycin, alanine, glutamic acid, asparagin, aspartic acid and cystine. Soya-bean meal and trypsin proved to enhance the activity of quite a number of organisms.

12. Antibiotic principle from *Moringæ pterygosperma* by Shri P. A. Kurup at the Indian Institute of Science, Bangalore.

The following was investigated :—

(1) The factors influencing the yield and the purity of pterygospermin preparations.

(2) Various methods of extraction in order to obtain optimum yields of pterygospermin.

(3) Methods of purification of pterygospermin.

(4) The analysis and degradation of pterygospermin and fixing a chemical structure for it.

(5) Its anti-bacterial and anti-fungal activity, as also its influence on germination of seeds (monocots and dicots).

(6) Its mode of action which includes the influence of growth factors on its action and also the effect of the anti-biotic on transaminating enzyme systems.

(7) Its *in vivo* protective action in experimental animals against staphylococcal and plague infections.

(8) Its pharmacological properties comprising of its toxicity in experimental animals, its effect on blood pressure and respiration and on the heart :

(9) Investigations of anti-bacterial action of substances chemically related to pterygospermin in order to shed light on the relationship of the structure with anti-bacterial action.

The general conclusions drawn are that pterygospermin is a low-melting crystalline substance of exceptional potency exerting its action on several Gram-positive and Gram-negative bacteria which include mycobacteria, and also on fungi. It does not significantly inhibit germination of seeds in low concentrations. It possesses low toxicity and shows no untoward pharmacological reactions. It protects experimental animals against *staphylococcal* infections but not against plague.

Chemically pterygospermin is related to benzyl-isothiocyanate and may be considered to possess certain structural similarity with penicillin. Interesting data have been collected by the investigation of related substances which may be of value in postulating the action of penicillin on a mechanistic basis.

It has been shown that pterygospermin exerts its action by interfering with the glutamic-acid transaminating enzymes.

13. Lipotropic activity of proteins with special reference to supplementary methionine by Kumari Vedam Jayem at the All-India Institute of Hygiene & Public Health, Calcutta.

As a preliminary study in following the conditions for production and dietary treatment of fatty livers, data had to be collected for the lipotropic factors in Indian foodstuffs. Methionine was taken for the purpose as lipotropic factor and some 44 Indian foodstuffs were analysed for the methionine content by the chemical method (Lavine, *Jour. Biol. Chem.*, 1943, **151**, p. 281) and the microbiological method (Horn, Jones, Blum, *Jour. Biol. Chem.*, 1946, **166**, pp. 321-26).

It was found that black-gram, ground-nut and broad bean had values varying from 112 mg. to 164 mg. per 100 g. It was decided to try the effect of supplementing the deficient diets with black gram, ground-nut and broad bean at ten per cent protein level, skim milk at 15 per cent level was used for comparison.

Fatty infiltration in liver of rats was produced by dietary means. A suitable diet adapted for this purpose in the laboratory contained casein five per cent, ground-nut oil 20 per cent, starch 50 per cent, glucose 20 per cent, salt mixture five per cent and supplements of 0.25 g. yeast daily and two drops of shark-liver oil once a week were given for the vitamins.

It will be seen that the diet given contains fat at 20 per cent level, much less than is recommended in many other diets for the production of cirrhosis of the liver. Even with the diet mentioned above it was possible in the laboratory to show that the livers of such rats in four weeks of experimentation had a fat content of 12.2 per cent and microscopic examination showed typical fatty infiltration.

The 'curative diets' were constituted in such a way that the addition made was any of the following : Ground-nut, black gram, broad bean, at 40 per cent level giving a protein content of ten per cent (*plus* five per cent casein) and methionine value varying from 267 mg. to 319 mg. per 100 g. of diet. The starch content was proportionately cut to include the high protein level. It was seen that by the end of four weeks of experimental period, when the rats were killed and the livers examined, there was regression in fat content of the liver by four to five per cent (by chemical analysis) and the histological picture was also indistinguishable from the normal. In one of the livers examined in the second week of feeding ground-nut supplement, regressive changes were visible, but not in the liver examined after one week of experimentation.

This experimentation serves to demonstrate the use of certain Indian foodstuffs in treating fatty livers in experimental animals.

14. Edible brackish-water bi-valves of South India and their bearing on human diseases by Shri K. C. Abraham at the King Institute, Guindy, Madras.

Brackish-water clams of the species *Meretrix casta* were fed on or inoculated with suspensions of cholera vibrio (Inaba strain) and the clams kept in the Laboratory in circulating brackish-water, to study the viability of the vibrios in the clams. Vibrios were isolated up to a maximum period

of three days from the clam meat. The recovered strains did not differ from the original in their agglutinative ability, biochemical reactions and hæmolytic activity. The vibrios did not multiply in the body of the clam as the steady fall in the number of colonies during the three days of isolation indicates. The available evidence suggests that it is improbable that these clams are responsible for maintaining cholera endemicity.

15. Carcinogens and the problem of cancer by Shri S. Duraiswami at the Indian Institute of Science, Bangalore.

20-methylcholanthrene in a colloidal suspension was found to increase the number of mutant sectors obtained in giant colonies of the control diploid brewery yeast. It was, however, observed that the alcohol used in introducing the carcinogen into the nutrient broth itself gave rise to 'background' mutations. Repeated attempts to induce tetraploidy with the aid of the chemical gave only negative results. Investigations on the effect of 20-methylcholanthrene on the rate of growth of the yeast showed that the chemical did not exert any marked effect. In an attempt to obtain a culture of yeast cells showing a uniform cytological behaviour, a necessary pre-condition to any successful study of the cytological effects of the carcinogen, the effect of (i) oxidative dissimilation, (ii) washing with M/15 KH_2PO_4 , and (iii) continuous æration for six hours of on the cytology of the diploid yeast, was followed using the Feulgen technique. The last mentioned appeared to be the most promising in this respect.

16. Rôle of corticoids in toxæmias of pregnancy by Dr. Dhirendra Kumar at the K. G. Medical College, Lucknow.

Evaluation of the rôle of the corticoids in toxæmias of pregnancy, assuming it to be a disease of adaptation, was undertaken by estimating the variations of sodium, chloride and potassium in blood as suggestive of mineral corticoid activity, while excretion of 17-ketosteroids in urine and total eosinophilic count in blood for gluco-corticoid. At the time when this work was started it was clear that the criteria mentioned above being an indirect one, is subject to variations arising from external factors, such as diet, but considering the practical limitations regarding the equipment available and other factors, these were the only methods possible to assay the corticoids in the said disease.

The above set of investigations was carried out, apart from actual cases of pre-eclampsia and eclampsia, in normal pregnancies during latter halves to find out the average normal variations. The same set of estimations was also undertaken in those normal and abnormal conditinos of pregnancy which pre-dispose to the syndrome of toxæmias of pregnancy. The Table shows the average normal values for sodium, chloride, potassium and total eosinophilic count in blood and 17-ketosteroids in urine in normal 60 primiparas at different periods of their advancing pregnancy during the latter half. A glance at the Table I at once points out one fact that no consistent correlation of these findings could be established as the pregnancy went on advancing. No evidence is there to note that sodium and chloride are retained during the latter half of pregnancy.

TABLE.

Period of pregnancy, in weeks	Number of patients	SODIUM		POTASSIUM		CHLORIDES		T. EOSN		17-KETO	
		Average	Min. Max.	Average	Min. Max.	Average	Min. Max.	Average	Min. Max.	Average	Min. Max.
24	6	290	270-310	20	18-22	600	620-640	363	210	-	-
28	6	282.6	272-2290	19.8	18.6-20	590	570-610	300	280-350	-	-
32	6	300	250-360	19.6	19-20	534	580-644	253	210-310	20.5	20-211
34	4	284	240-328	20.5	20-21	570	580-590	320	300-340	-	-
36	10	282.6	260-318	19.03	18-20.1	600	580-610	260	210-315	18.5	16-19.5
38	12	300	260-320	19.5	17.5-22	585	580-590	320	300-340	-	-
40	16	278.6	252-300	19.5	18-20	580	560-595	260	210-340	17.5	15.1-20

Regarding the normal and abnormal conditions of pregnancy which pre-dispose to pre-eclampsia and eclampsia the following types of cases were investigated. In all these cases at least three to four sets of investigations were performed, usually two before the delivery and one or two after it.

1. Elderly primigravida	6
2. Multiple pregnancy	5
3. Hydramnios	9
4. Patient with previous history of eclampsia	1
5. Acute yellow atrophy	1

In all these cases no significant increase was noted in sodium and chloride and fall of potassium in blood as suggestive of hyper-mineral-corticoid activity. This can be explained on the basis that no clinical features were noted in these cases showing any mild degree of pre-eclampsia. In post-partum period again no fall of sodium and chloride was noted except in all the cases of elderly primigravidas.

In the group of toxæmias of pregnancy following cases were investigated :

1. Essential hypertension	9
2. Pre-eclampsia	13
3. Eclampsia	1

Cases of essential hypertension did not reveal any significant rise or fall in the levels of sodium, chloride, potassium and total eosinophilic count in blood, and in the excretion of 17-ketosteroids in urine.

According to the criteria employed no hyper-mineral-corticoid activity was noted in actual cases of pre-eclampsia and eclampsia. No consistent high values were noted in sodium and chloride levels in blood except in few cases in ante-natal period. On the contrary, in some cases the above electrolytes were found to be low when compared with the post-natal findings even after excluding any possibility of post-partum pre-eclampsia. These findings are in agreement with the recent work of Nordenstrahl (1952).

As was expected that potassium level will be lowered in these cases demonstrating hyper-mineral corticoidism, it was found to be more or less constant in all the above patients. This finding also supports the work done by Dieckmann (1942), Mukherjee and Govan (1950), and Nordenstrahl (*loc. cit.*). The latter writer, although agreeing with the above finding, has demonstrated that potassium on the contrary is retained in these cases the site of retention being intracellular in musculature and brain.

The only partial evidence noted in favour of its being a disease of adaptation is the diminished excretion of 17-ketosteroids during the active phase of this disease. Other workers who have supported this finding are Collard and Heusgham (1950) and Devis and Devis Vanden Ekhoudt (1950).

Be that as it may, according to the criteria employed to evaluate the hyper-activity of mineral corticoid it is still not possible to rule out the probability of its rôle in the syndrome of toxæmias of pregnancy. Other workers in this field are of the opinion that no appropriate method is available for micro-bioassay of the effect of steroids in mineral metabolism, and the diseases of adaptation have been handicapped principally due to the above fact. Chromatographic study of adrenal cortical hormones by Burton *et al.* (1951) and development of a highly accurate procedure applicable to investigate the mineral activity (Tait, Simpson and Grundy, 1952) and further separation of a 'salt-retaining factor' which has by far the greater part of the salt-retaining activity of the whole-beef adrenal-gland extract by the latter workers have shown promising results in this field. In the light of the above studies it seems quite reasonable to reconsider the rôle of hyper-activity of the mineral corticoids in diseases of adaption in general and toxæmias of pregnancy in particular.

V. THE INDIAN JOURNAL OF MEDICAL RESEARCH AND INDIAN MEDICAL RESEARCH MEMOIRS.

1. (a) *The Indian Journal of Medical Research, Volume XL, 1952.*—The usual four numbers of the *Journal* were published and these appeared in January, April, July and October, respectively. All these numbers appeared much later than their scheduled dates, i.e. January number in July, April number in August, July number in December 1952 and October number as late as May 1953. This inordinate delay was due to labour trouble, strikes amongst press workers and internal disputes between the Publishers and Printers. The average size of each number was 154 pages and tabular matter with six plates.

(b) *The Memoirs.*—No *Memoir* was published with Volume XL.

2. (a) *The Indian Journal of Medical Research, Volume XLI, 1953.*—All the four numbers for volume XLI are still with the Press. The January 1953 number is expected to be out shortly, while the April 1953 number may be expected to be out by the middle of November 1953. These two numbers will contain 142 pages and five plates, and 162 pages and 12 plates, respectively. The July and October 1953 numbers are under print and these are expected to be out early in November and December 1953, respectively. These will consist of 100 pages and eight plates, and 106 pages and three plates, respectively.

(b) *The Memoirs.*—No *Memoir* will be published with Volume XLI.

VI. INDIAN JOURNAL OF MALARIOLOGY.

Indian Journal of Malariology, Vol. VI, Nos. 2, 3 and 4, 1952.—The June, September and December 1952 issues comprising 86, 136 and 146 pages each, representing 368 pages of printed and tabular matter, were published during the year under report.

The March 1952 issue, publication whereof was reported in the last year's note, contained 132 pages. Thus, Volume VI of the *Journal* comprised 500 pages of printed and tabular matter.

Besides contributors in India, the *Journal* continues to enjoy the confidence of research workers in the foreign countries as well. Out of 41 papers comprising Volume VI, four papers were submitted by workers in Trinidad, U.S.A. and England.

Indian Journal of Malariology, Volume VII, 1953.—The March 1953 issue comprising 92 pages has been published.

The June 1953 issue covering 130 pages is at the page-proof stage and is likely to be out of the press very shortly.

The September 1953 issue is also in the press.

Distribution.—Requests from institutions in India and abroad are received regularly for inclusion in the Exchange Mailing List, and where the literature published by the parties is considered sufficiently useful, such requests are invariably entertained. The table shows the distribution of the *Journals* :—

Subscribers, as per list for Volume VI (Year 1952):

Foreign countries	70	}	168
In India	98		
Exchange list		114
Free list		109
Voucher copies to advertisers, specimens, propaganda, etc.					25
The Malaria Institute of India		64
Balance held by the publishers for sale		120
					<hr/> 600 <hr/>

VII. MICROFILM AND PHOTOCOPY SERVICE UNITS.

(a) At the Central Research Institute, Kasauli.

During the year under review 1,763 photostat copies, 244 microfilm copies and six photo-micrographs were supplied to medical colleges, research institutions, etc., in the country.

(b) At the Indian Cancer Research Centre, Tata Memorial Hospital, Bombay.

During the year under review 2,445 pages of the microfilm copies were supplied to various institutions in the country.

The Units can supply microfilm and photostat copies of articles from medical and scientific publications available in libraries in India. The photostat copies can be read as easily as any printed matter without the aid of any special equipment, whilst an equipment called "Reader" is required for reading microfilm copies.

The rates for the supply of microfilm and photostat copies are :—

<i>Microfilm :</i>	Rate
	Rs. a. p.
For the first ten pages or less ...	3 0 0
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Size up to 11" × 6" ...	0 8 0
„ „ 17" × 11" ...	0 12 0
„ „ 23" × 17" ...	1 4 0

VIII. PAPERS PUBLISHED DURING THE YEAR 1953. .

A. In the Indian Journal of Medical Research :

1. AHMAD, K., KARIM, M. A. and DE, H. N.—Choline Contents of some Common Bengal Foodstuffs (Vol. 41, No. 4).
2. AHUJA, M. L., and SURI, J. C.—A Brief Note on a New Technique for Supply of Diagnostic Sera (Vol. 41, No. 3).
3. ANAND, B. K.—Effect of Temperature on Liberation of Acetylcholine (Vol. 41, No. 3).
4. BHASKARAN, K.—Studies on Vibrio Dissociation. Part I. Smooth Rough Dissociation of *V. cholerae* (Vol. 41, No. 2).
5. CHAKRAVARTY, M. L., and SEN, S. K.—Distribution of Serum Phosphatases in Health and Diseases (Vol. 41, No. 2).
6. CHAKRAVARTY, N. K., CHAKRAVARTI, H. S., DUTTA, B. N., and CHAUDHURI, R. N.—Body-fluid Changes in Epidemic Dropsy (Vol. 41, No. 3).
7. CHATTOPADHYAY, H., and BANERJEE, S.—Effect of Germination on the Biological Value of Proteins and the Trypsin-inhibitor Activity of some Common Indian Pulses (Vol. 41, No. 2).
8. DE, N. K.—The Alleged Toxicity of Indian Pulses (Vol. 41, No. 3).
9. DEB, C., and BANERJEE, S.—Hæmatological Studies in Normal and Scorbutic Guinea-pigs (No. 41, Vol. 1).
10. DUTTA, N. K., and TAMHANE, R. G.—Effect of Atropine, Pethidine, Procaine, Quinidine and Prophen Pyridamine Maleate on the Histamine-induced Gastric Acidity in Cats (Vol. 41, No. 1).
11. GOTHOSKAR, S. S., and SREENIVASAN, A.—Biotin Metabolism in Micro-organisms. Part I. Biosynthesis (Vol. 41, No. 1).
12. GOTHOSKAR, S. S., and SREENIVASAN, A.—Biotin Metabolism in Micro-organisms. Part II. Rôle of Biotin in some Oxidative Systems (Vol. 41, No. 1).
13. GOTHOSKAR, S. S., and SREENIVASAN, A.—Biotin Metabolism in Micro-organisms. Part III. Aspartic-acid Oxidation and Deamination (Vol. 41, No. 1).
14. GOVINDAN NAYAR, K. N.—An Apparatus for Starling's Heart-lung Preparation (Vol. 41, No. 1).
15. GUARD, H. R., and BHENDE, Y. M.—Changes due to Ageing in the Abdominal Aorta (Vol. 41, No. 2).
16. GUPTA, K. C., and CHOPRA, I. C.—Synergistic Action of Streptomycin and Chloromycetin : *In vitro* Studies (Vol. 41, No. 4).
17. GUPTA, K. C., and CHOPRA, I. C.—*In vitro* Studies of Combined Action of Chloromycetin and Ptergospermin (Vol. 41, No. 4).
18. GUPTA, K. C., CHOPRA, I. C.—Tuberculostatic Activity of *Leea hirta* Roxb. (*Kaka Jangan*) (Vol. 41, No. 4).
19. GUPTA, K. C., CHOPRA, I. C.—A Short Note on Anti-bacterial Properties of Chaksine : An Alkaloid from *Cassia absus* Linn. (Vol. 41, No. 4).

20. GUPTA, N. P., and MANGALIK, V. S.—Plasma Cells and Antibody Production (Vol. 41, No. 3).
21. GURKIRPAL SINGH, and AHUJA, M. L.—Observations on the Intestinal Epithelium Desquamating Enzyme of Vibrios isolated from Cholera and Non-cholera Sources (Vol. 41, No. 3).
22. HIRWE, RATNAPRABHA and MAGAR, N. G.—Effect of Autoclaving on the Nutritive Value of Pulses (Vol. 41, No. 2).
23. INDAR JIT.—The Development of the Goblet Cells in the Human Gut (Vol. 41, No. 4).
24. JOSHI, S., MASTER, F. and MAGAR, N. G.—Nutritive Value of some Bombay Fish (Vol. 41, No. 4).
25. KAMATH, G. G., and MAGAR, N. G.—Ultra-violet Absorption Characteristics of some Indian Shark-liver Oils (Vol. 41, No. 3).
26. KARKUN, J. N. and MUKERJI, B.—Studies on Chromatophoretropic Hormone of the Pituitary Gland. Part III. The Influence of Melanophore Hormone upon the Synthesis of Melanin Pigments in the Skin of Frogs (*Rana tigrina*) (Vol. 41, No. 4).
27. KOTHARE, S. N. and SEN, P. K.—Healing of the Myocardial Wound at the Site of Auricular Appendectomy in Dogs (Vol. 41, No. 3).
28. LAL, B. M. and RAJAGOPALAN, R.—Studies on Mutual Supplementation in Vegetable Proteins (Vol. 41, No. 2).
29. MANMOHAN SINGH, GURBACHAN SINGH, and KAPOOR, S. P.—Comparative Study of Four Serological Tests for Syphilis (Vol. 41, No. 2).
30. MANMOHAN, SINGH, KHANNA, SUKH DEV and CHADDAH, R. R.—*In vitro* Sensitivity of *Salmonella typhi* to Four Antibiotics (Vol. 41, No. 4).
31. NARAYANAN, E. K. and BHATIA, A. L.—Nephelometric Determination of Bacterial Vaccine (Vol. 41, No. 3).
32. NARAYANAN, E. K., DEVI, P. and MENON, P. S.—Enzymes of *V. cholerae* with Possible Rôle in Pathogenesis (Vol. 41, No. 3).
33. NATH, M. C. and CHAKRAVARTI, C. H.—Studies on the Effect of Aceto-acetate and β -hydroxybutyrate on Vitamin B₁, both *in vivo* and *in vitro* (Vol. 41, No. 2).
34. PATHAK, J. D., PAI, M. L. and GANDHI, A. M.—Gastric Response, Digestion and Evacuation Time of some Milk Preparations. Part I of the Series (Vol. 41, No. 1).
35. PENDSE, G. S., DANDEKAR, V. M., and BHAVE, L. S.—The Study of the State of Refraction of one Indian Community, with Special Reference to Myopia (Vol. 41, No. 1).
36. PILLAY, K. V. and PILLAI, S. S.—Studies in the Purification of Anti-smallpox Vaccine (Vol. 41, No. 1).
37. RAO, K. SOMESWARA, DE, N. K. and RAO, D. SUBBA.—Investigation of an Outbreak of Night-blindness in a Village near Madras (Vol. 41, No. 3).
38. RAO, S. SHANTA and LAHIRI, D. C.—Studies on Changes associated with Conversion of Diphtheria Toxin into Diphtheria Toxoid with Formalin and Heat (Vol. 41, No. 1).

39. RATNAVATI, C.—Evaluation of the Cytological Test in the Diagnosis of Cancer of the Uterus (Vol. 41, No. 3).
 40. ROY, B. R.—Physiological Suitability of Ethyl Gallate as an Antioxidant. Part I. Acute Toxicity Dose (Vol. 41, No. 2).
 41. ROY, B. R.—Physiological Suitability of Ethyl Gallate as an Antioxidant. Part II. The Determination of Chronic Toxicity (Vol. 41, No. 2).
 42. SATHE, VANAMALA and KRISHNAMURTHY, K.—The Effect of Organic Acids on the Availability of Iron (Vol. 41, No. 4).
 43. SATHE, VANAMALA and KRISHNAMURTHY, K.—Phytic Acid and Absorption of Iron (Vol. 41, No. 4).
 44. SHIV KUMAR and LAL, SURENDRA K.—Some Toxic Metabolic Factors concerned in the Development of *Diabetes mellitus* in the Albino Rat (Vol. 41, No. 2).
 45. SHRIVASTAV, J. B.—A Survey of the Intestinal Parasites in the Human Population in Bombay, with Special Reference to *Endamæba histolytica* (Vol. 41, No. 4).
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 47. SIRSAT, M. V.—Cancer in Infancy and Childhood (From Birth to 14 Years of Age) (Vol. 41, No. 2).
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 50. SUBRAHMANYAN, K. and MAJUMDAR, N.—Environmental Conditions in Flour Mills (Vol. 41, No. 1).
 51. SURI, J. C.—The Use of Proskauer and Beck's Liquid Medium in the Laboratory Diagnosis of Tuberculosis (Vol. 41, No. 1).
 52. VENKATARAMAN, R., and SREENIVASAN, A.—The Bacteriology of Fresh-Water Fish (Vol. 41, No. 4).
 53. VENKATESWARLU, P., RAMANATHAN, A. N. and RAO, D. NARAYANA.—A Direct Volumetric Procedure for Estimation of Thorium and its Application in the Preparation of Thorium Reagent for Micro-estimation of Fluorine (Vol. 41, No. 2).
 54. VENKATESWARLU, P. and RAO, D. NARAYANA.—Investigations on the Removal of Fluoride from Water. Rapid Removal of Fluorides with Magnesium Oxide (Vol. 41, No. 4).
 55. WAHI, P. N. and NIGAM, R. G.—Paper-partition Chromatography of Amino Acids (Vol. 41, No. 4).
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B. INDIAN JOURNAL OF MALARIOLOGY, Vol. VI:—

1. BHOMBORE, S. R., BROOKE WORTH, C., and NANJUNDIAH, K. S.—A survey of the economic status of villagers in a malarious irrigated tract in Mysore State, India, before and after DDT residual insecticidal spraying (Vol. 6, No. 4).
2. BROOKE WORTH, C. and SITARAMAN, N. L.—Studies on the bionomics of *Anopheles fluviatilis* James, 1902, in Mysore State, India. I. Review of the literature on bionomics of *A. fluviatilis* (Vol. 6, No. 4).
3. JASWANT SINGH, KRISHNAN, K. S. and DAVID, A.—Natural infection of *P. relictum* in weaver bird, the baya (*Ploceus philipinus*) (Vol. 6, No. 4).
4. JASWANT SINGH, KRISHNASWAMI, A. K. and RAMAKRISHNAN, S. P.—The distribution of human plasmodia in India (Vol. 6, No. 4).
5. JASWANT SINGH, RAMAKRISHNAN, S. P., KRISHNASWAMI, A. K., SATYA PRAKASH, MAMMEN, M. L. and RAY, A. P.—Drug resistance of pre-erythrocytic forms of *Plasmodium gallinaceum* Brumpt, 1935 (Vol. 6, No. 4).
6. JASWANT SINGH and RAY, A. P.—Studies on experimental mixed infections in simian malaria (Vol. 6, No. 4).
7. JASWANT SINGH, RAY, A. P., BASU, P. C. and MISRA, B. G.—Effect of pyrimethamine in human malaria. Part I (Vol. 6, No. 4).
8. JASWANT SINGH, RAY, A. P., MISRA, B. G. and BASU, P. C.—Effect of pyrimethamine in human malaria (*P. falciparum*). Part II. (Vol. 6, No. 4).
9. RAMAKRISHNAN, S. P., RAY, A. P., MENON, M. K. and BHATNAGAR, V. N.—A note on the effect of proguanil on the sporogony cycle of *P. gallinaceum* Brumpt, 1935. (Vol. 6, No. 4).
10. RAMA RAO, R. and GIRI, K. V.—Free amino acid pattern of blood of normal and malarial chick infected with *P. gallinaceum* (Vol. 6, No. 4).
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12. SRIVASTAVA, R. S. and CHAKRABARTI, A. K.—Malaria control measures in the Terai area under the Terai colonization scheme, Kichha, District Nainital : 1949 to 1951. (Vol. 6, No. 4).
13. TYSSUL-JONES, T. W.—DDT as a mosquito larvicide and its application in high spreading oils on large expanses of water sheets. (Vol. 6, No. 4).
14. VEDAMANIKKAM, J. C.—Seasonal variation in the breeding places of *Anopheles fluviatilis* James in Wynaad and its relationship to eradication of the species (Vol. 6, No. 4).

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16. BROOKE WORTH, C.—Notes on the anopheline fauna of a hill tract in Mysore State, India (Vol. 7, No. 2).
17. DAVID, A. and KRISHNAN, K. S.—Some observations on experimental infections in domestic pigeons (*Columba livia* Gmelin) with *P. relictum* (Vol. 7, No. 2).
18. GRAMICCIA, G. and SACCA, G.—Notes on kala-azar and its control in Iswarganj Thana, Mymensingh District, East Bengal (Vol. 7, No. 1).
19. IYENGAR, M. O. T., MATHEW, M. I. and MENON, M. A. U.—Malaria in the Maldiv Islands (Vol. 7, No. 1).
20. JASWANT SINGH, MISRA, B. G. and RAY, A. P.—Effects of pyrimethamine in human malaria (suppressive treatment). Part III (Vol. 7, No. 1).
21. JASWANT SINGH, MISRA, B. G. and RAY, A. P.—Suppressive treatment with amodiaquin (Vol. 7, No. 1).
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23. JASWANT SINGH, NAIR, C. P. and RAY, A. P.—Comparative study on 4-aminoquinolines against *P. cynomolgi* in rhesus monkeys (Vol. 7, No. 3).
24. JASWANT SINGH, NARAYANDAS, M. G. and RAY, A. P.—Assay of antimalarials against the sporogony cycle of *P. gallinaceum* (Vol. 7, No. 1).
25. JASWANT SINGH, RAY, A. P. and CHANDRASEKHAR, G. R.—Screening of antimalarials against *P. gallinaceum* in chicks. Part II (Vol. 7, No. 2).
26. JASWANT SINGH, RAY, A. P. and MISRA, B. G.—4-aminoquinolines in the single dose treatment of malaria (Vol. 7, No. 1).
27. JASWANT SINGH, RAY, A. P., MISRA, B. G. and BASU, P. C.—Toxic manifestations of repeated doses of pyrimethamine in rhesus monkeys. (Vol. 7, No. 3).
28. JASWANT SINGH, RAY, A. P. and NAIR, C. P.—J.S.B. stain—Its preparation in the powder form and the staining technique (Vol. 7, No. 3).
29. KRISHNASWAMI, A. K., SATYA PRAKASH, BAMI, H. L. and RAMAKRISHNAN, S. P.—Studies on *Plasmodium berghei* n. sp. Vincke and Lips, 1948. XIV. Reaction of blood-induced infection in albino mice to proguanil for dihydrotriazine metabolites (Vol. 7, No. 3).
30. KRISHNASWAMI, A. K., SATYA PRAKASH and RAMAKRISHNAN, S. P.—Studies on *Plasmodium berghei* n. sp. Vincke and Lips, 1948. XII. Attempts to estimate *in vivo* the acquired immunity in albino rats (Vol. 7, No. 2).
31. RAMAKRISHNAN, S. P.—Studies on *Plasmodium berghei* n. sp. Vincke and Lips, 1948. VIII. The course of blood-induced infection in starved albino rats (Vol. 7, No. 1).

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PAL, R. and SHARMA, M. I. D.—Rapid loss of biological effectiveness of DDT applied to mud surfaces, p. 251.

PAL, R. and SHARMA, M. I. D.—Micro-bioassay method for DDT, p. 275.

PAL, R. and SHARMA, M. I. D.—Behaviour of mosquitoes in relation to insecticidal applications, p. 281.

PAL, R., SHARMA, M. I. D. and KRISHNAMURTHY, B. S.—Studies on the development of resistant strains of houseflies and mosquitoes, p. 303.

SHARMA, M. I. D. and PAL, R.—Comparative field studies on the residual effectiveness of DDT, BHC, DDT and BHC combined spray and dieldrin against mosquitoes, p. 317.

PAL, R.—Dieldrin for malaria control, p. 325.

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